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IMMUNIZATION OF HUMAN BEINGS WITH ORAL LACTOBACILLI[1]

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A possible relationship between intradermal vaccination of human beings with heat-killed oral lactobacilli and an effect on the average numbers of lactobacilli in the saliva was described in a preliminary report by Canby and Bernier (1). Repeated intradermal vaccination led to an increase in the blood agglutinin titers for the strains of lactobacilli used in the vaccines. Comparisons of the average salivary counts for lactobacilli, before and after vaccinations, indicated that there had been a reduction of the averages in 19 out of 20 persons after vaccination. The authors proposed that the lactobacilli in the saliva had been affected by a substance, having growth inhibitory properties, which was stimulated by vaccination.

This report presents the findings in a similar type of study. Our purpose was to determine whether any increase in the blood agglutinin titers, for the strains in the vaccines, could be associated with a decrease in the salivary counts for lactobacilli.

METHODS

Experimental Groups. Volunteers were selected on the basis of the averages from 4 separate salivary counts for lactobacilli over a period of 2 weeks. Saliva was collected on arising in the morning by softening sterile paraffin in the mouth for 2 minutes, then chewing vigorously for 3 minutes and expectorating the stimulated saliva into a sterile tube. Dilution and platings were begun within 2 to 3 hours after collection. Saliva was diluted so that at least 100 lactobacillus colonies were present on the plates to be counted. It was necessary to plate several dilutions of each sample in order to maintain this standard. Counts were made after spreading 0.1 cc. of the mixed, diluted saliva on Petri plates containing Kulp's tomato agar pH 5.0 (2) and incubating 2 to 3 days at 37°C. The representative colonies were counted under a low power microscope. Persons with counts exceeding 40,000 lactobacilli per cc. of saliva were selected for the group. Half of these were to serve as controls.

This report includes 20 of the experimental group and 13 controls whose records were complete. Salivary counts of the type described were repeated 16 to 32 times at similar time intervals in the 2 groups throughout 4 months

[1] Read at the 22nd General Meeting of the International Association for Dental Research, Chicago, Illinois, March 18–19, 1944 (J. D. Res., **23**: 196, 1944). Received for publication September 3, 1944.

[2] Captain, Dental Corps, A.U.S. No objection to publication on grounds of military security and policy, Bureau of Public Relations, War Department.

of the study, and again 6 to 10 times over a period of 5 weeks about 2 months later.

Dental Examinations and X-rays. The teeth of the entire group were examined by the use of an explorer and mirror. Records were made of all carious areas, fillings, prostheses and missing teeth. Bite-wing and anterior radiographs were employed to supplement clinical findings. The group was requested not to have dental work done during the course of the experiment.

Antigens and Agglutination Tests. Lyophilized (3) stock cultures were suspended in sterile 0.85% saline, inoculated onto tomato agar slants pH 6.1 (2) and incubated anaerobically for 3 to 4 days at 37°C. The growth on the slants was harvested in sterile phosphate buffered saline, pH 7.0, pooled and washed 3 times in the sterile buffered saline.

Suspensions were standardized in a Klett-Summerson colorimeter to a reading of 112 to 114. The antigens were prepared for agglutination by heating the suspensions in a water bath at 60°C for 30 minutes, and adding merthiolate as preservative (final dilution 1:10,000).

All persons were bled by venipuncture prior to vaccination as well as 2 weeks after the last injection. Further bleedings were made at intervals of 1, 2 and 5 months after the completion of vaccination.

The agglutination tests on sera obtained prior to injections were made with dilutions of serum ranging between 1:2 and 1:512. After vaccination the sera were tested in dilutions between 1:40 and 1:10240. Each serum was tested with each of the two strains used in the vaccines.

Vaccines. The strains were chosen from the results of cross agglutination tests between 40 stock lactobacillus antigens and their immune rabbit sera. The greatest degree of cross-reactivity was demonstrated by strains 4 S and 13 C. The colonial and morphological characteristics of 4 S were similar to Hadleys' Type I, while 13 C was similar to her Type II (4). The suspensions for use as vaccines were prepared in the same manner as the antigens described above, except that living as well as heat-killed concentrated vaccines in 2 densities were made from each strain. The concentrated living vaccines contained about 900 million and 2000 million lactobacilli per cc. respectively (Klett-Summerson readings 202 and 395); similar suspensions were made with heat-killed lactobacilli. Vaccines of 4 types were prepared from the concentrated suspensions by mixing *equal portions* of the 4 S and 13 C strains so that the final approximate content of organisms, in millions per cc., was as follows:

	Heat-killed		*Living*
#1	450—4 S 450—13 C	#3	450—4 S 450—13 C
#2	450—4 S 1000—13 C	#4	450—4 S 1000—13 C

Vaccination. Preliminary comparisons between the intradermal and subcutaneous routes of inoculation in human beings and animals (rabbits and guinea pigs) demonstrated that a greater increase in agglutinin titers could be obtained when the subcutaneous route was employed. Other animal tests indicated

that higher agglutinin titers may result if the living vaccine is given simultaneously with the heat-killed. Persons in the group to be vaccinated submitted to skin tests with the vaccines in order to eliminate any possibility of hypersensitivity, injections of 0.1 cc. being made intradermally on opposite forearms, using vaccines #1 and #3. Vaccinations were begun after observation of the reactions to skin tests and when salivary counts had been completed over a period of at least one month. The course of injections consisted of 4 subcutaneous inoculations at weekly intervals, the living and heat-killed vaccines being given simultaneously in the deltoid region of opposite arms. Living vaccine was always used in the right arm and heat-killed in the left. The first 3 injections consisted of 0.5, 1.0 and 1.0 cc. of vaccines #1 and #3, while the 4th was 1.0 cc. of vaccines #2 and #4. Each arm received a total of 3.5 cc. of the living and heat-killed vaccines respectively and each person received 7 cc. of vaccine totaling about 7,400 million lactobacilli.

Controls. The group of 13 controls was treated in the same manner as the vaccinated in so far as salivary counts and agglutination tests were concerned, but no injections of any type were given.

EXPERIMENTAL RESULTS

The intradermal skin tests with vaccines #1 and #3 were positive in all cases. Pink, flat, areas of erythema, which varied from 5 to 40 mm. in diameter were first visible in from 30 minutes to 10 hours. After 24 to 48 hours the responses had developed into either small flat discolored areas or small nodules which measured about 5 mm. in diameter; both gradually faded over a period of 4 to 6 weeks. The observed reactions were due mainly to local irritation caused by the vaccines and were not considered as being of the hypersensitive type.

In 6 to 18 hours after the first subcutaneous injections with 0.5 cc. of #1 and #3 vaccines, pink, tender, warm areas of erythema were apparent at the sites of inoculation. In 24 hours the areas were hardened and tender to touch, or to flexure and rotation of the arm. The responses ranged from nil to 7 cm. in diameter, and the following day (48 hours) were reduced in size, intensity, and tenderness. They faded completely by the time the second injections were due, except in 2 cases which lasted another week. The same type, but slightly larger areas followed the 2nd and 3rd vaccinations, each consisting of 1.0 cc. of #1 and #3. The diameters ranged from 1–10 cm. after the 2nd subcutaneous injections, while responses to the 3rd measured from 3 to 9 cm. in diameter. Visible or palpable evidence of these areas was not present after 1 week. The final injections, made with 1.0 cc. of #2 and #4 vaccines elicited reactions which were similar to those following the initial subcutaneous vaccinations. These responses disappeared within 3 to 7 days. Neither general, axillary, nor local complications were observed or reported. No outstanding differences were observed between the type of responses to living and heat-killed vaccines. The results suggest that the organisms in the vaccines were easily disposed of by the subcutaneous tissues without severe reactions of any type.

All persons were bled prior to the vaccinations as well as 2 weeks, 1, 2 and 5

months after the last injections. The sera were tested for agglutinins in separate serological tests with 4 S and 13 C antigens. The average titers of the experimental and control groups are shown graphically in *figs. 1, 2* and *3*. It will be noted that the titers increased during vaccination and reached a maximum of around 1:600 and 1:700 at the 2 weeks interval. The titers for strain 4 S were consistently higher than those for 13 C in spite of the fact that there were more organisms of the latter strain in the total amount of vaccine. Sera obtained at later intervals were found to have progressively lower titers until at 5 months the levels were slightly higher or nearly the same as before vaccination. The agglutination tests indicate that the subcutaneous vaccinations caused an increase in the blood agglutinin titers for the 2 strains of lactobacilli employed

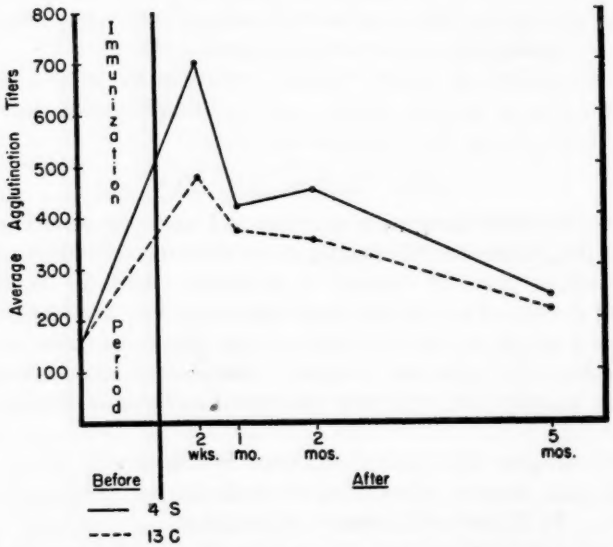

Fig. 1. Average agglutination titers of persons having reductions in average salivary counts which were greater than could be accounted for on basis of chance.

in the vaccines. The average titers in the control group varied only a little during the experimental period.

Pre-breakfast salivary samples were received and counts made for lactobacilli twice weekly during the 4 weeks preceding the injections, the 4 weeks of immunization and the 8 weeks post-immunization period. The counts for all persons were divided into 2 parts, those obtained up to and through the final injections were regarded as "before," all others within the 2 month period after the last injection as "after." The counts in each group, before and after, were added and averaged. If humoral antibodies, measured here as agglutinins, or some other substance stimulated by vaccination, have any effect on the lactobacilli in the mouth they might be presumed to be most effective when the blood titer is at its maximal or after completion of injections. It was noted

(*figs. 1* and *2*) that the average titers were highest at the 2 weeks period. All counts after the last injection may be regarded as having been affected by the

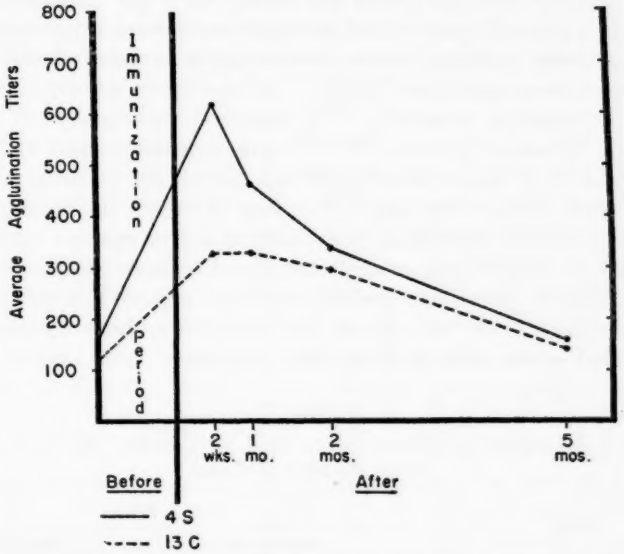

Fig. 2. Average agglutination titers of persons having changes in average salivary counts which were within limits of chance variation.

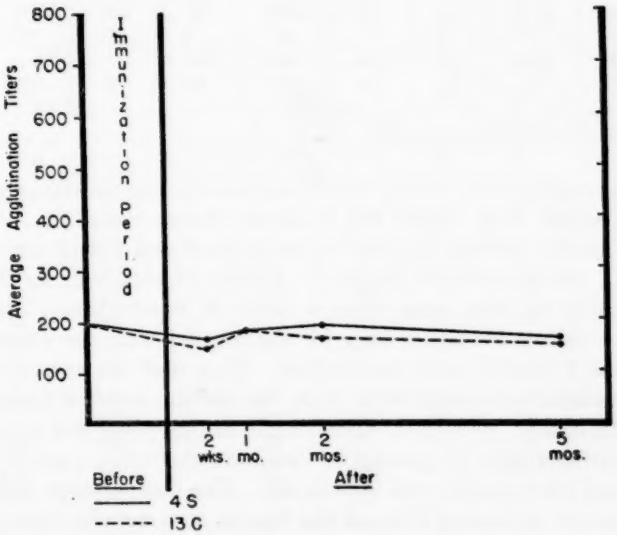

Fig. 3. Average agglutination titers of unvaccinated controls.

increased agglutinin titers. The counts in the control group were separated and averaged on the same time basis as used for the immunized group.

Salivary counts of the pre-breakfast type were repeated twice weekly for 5 weeks about 5 months after the final injections. The figures were averaged and compared with the averages before and during the 2 month interval after vaccination. It is generally agreed that comparisons between arithmetical averages have no significant meaning unless some analysis is made of the data which have been used to compute the averages. All changes in salivary counts whether they were increases or decreases, were analyzed statistically. The standard error of any difference between the averages was determined and applied to probability tables in order to estimate the percentage of times the observed differences could be due to chance (5). Any difference in the averages which was shown to have a percentage probability of 5% or less was regarded as significant, that is, a difference greater than could be accounted for on the basis of chance. Higher percentage probabilities were regarded as within the limits of chance variation. On the basis of the results from these statistical analyses the vaccinated group was divided into 2 sections. One showed significant

TABLE I

Persons having reductions in salivary counts which were greater than could be accounted for on the basis of chance

	BEFORE			AFTER					
				Two months			Five months		
Vol.	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest
ARA	88*	219	36	30	160	4	215	343	106
MAN	1424	6100	195	173	320	25	238	508	20
REE	814	2500	7	13	18	5	29	33	25
ROS	725	2300	25	245	540	109	—	—	—
SCO	161	434	26	56	127	13	56	147	23

* All figures given are in thousands.

decreases in average counts during the 2 months after vaccination, and the other had changes which were within the limits of chance variation. The before, after and 5 month averages as well as the highest and lowest counts for the former section are presented in Table I. It may be assumed, for the present, that the findings in these cases were a result of vaccination. The averages obtained from counts 5 months after were compared with the averages before and during the 2 months after vaccination. Note that the averages are lower than before vaccination except for A R A, but are the same or higher than the averages at 2 months. The observations might indicate that due to the decrease in blood agglutinins with the passage of time, as seen in *figs. 1* and *2*, there also was a decreased effect on the oral lactobacilli. The comparisons with the other averages, however, indicated that all the figures at 5 months were within the limits of chance variation.

The results of similar analyses for all other persons in the vaccinated group are shown in Table II. The persons in this section had changes in average

counts, increases or decreases, which were within the limits of chance variation. Figures on DIE, PER and WAT could not be obtained at 5 months.

TABLE II

Persons with changes in counts within limits of chance variation

	BEFORE			AFTER					
				Two months			Five months		
Vol.	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest
AND	118*	386	4	106	254	35	29	64	4
BRA	562	4000	53	332	892	202	426	668	118
CAR	722	6112	16	87	254	11	26	56	2
CUR	2256	9840	80	295	508	100	373	420	217
DIE	49	135	4	53	81	5	—	—	—
GRE	169	480	15	147	381	30	69	212	8
GOL	105	180	24	161	381	31	38	95	16
HAI	411	1588	2	8	25	2	4	8	2
KAU	69	127	16	80	145	32	56	184	1
KLE	5774	13580	1000	6223	10160	2500	2600	3360	2000
LEY	238	812	40	45	101	7	56	110	7
SUB	744	5000	11	311	761	80	1140	2540	200
MAR	660	1271	24	46	190	10	28	57	12
PER	91	728	36	50	181	24	—	—	—
WAT	299	524	13	309	1016	60	—	—	—

* All figures given are in thousands.

TABLE III

Controls—any changes were within limits of chance variation

	BEFORE			AFTER					
				Two months			Five months		
Vol.	Average	Highest	Lowest	Average	Highest	Lowest	Average	Highest	Lowest
AHA	14*	27	8	10	15	2	4	9	5 hundred
BUR	48	133	12	68	81	15	—	—	—
CAH	670	2869	59	533	254	34	51	127	14
FRA	299	734	90	333	1164	200	—	—	—
GUY	20	64	3	16	50	2	10	15	6
HEL	60	294	3	37	127	10	15	23	9
JOH	79	254	21	54	127	10	—	—	—
LAM	12	17	2	9	13	6	—	—	—
PAY	55	194	2	38	76	11	26	35	15
STO	131	327	26	147	386	46	151	343	31
SUS	298	899	20	233	992	25	29	76	10
VOG	313	576	23	259	508	118	—	—	—
PAR	95	533	6	97	512	5	—	—	—

* All figures given are in thousands.

As a summary of Tables I and II, 15 out of the 20 persons had reductions in counts during the 2 months after vaccination when the averages were compared

with the "before" average counts. Of the 15 persons having reductions, the differences in the averages of only 5 could be shown to be greater than could be accounted for by chance. All other reductions and increases were within the limits of chance variation. The data indicates that the counts in only 5 out of 20 persons or (25%) could have been affected by the vaccination.

The average agglutinin titers of the groups in Tables I and II are presented in *figs. 1* and *2* respectively. The curves are similar in shape and the average titers do not differ greatly. The group, whose counts were found to be significantly reduced, did not demonstrate humoral agglutinins in an appreciably greater amount than those whose reduction in counts were within the limits of chance variation. Persons with increases in average salivary counts, which were within the limits of chance variation were found to also have increased agglutinin titers. The latter were of no less magnitude than those of the persons with decreases. The results in this study seem to indicate that no direct relationship can be established between the increased agglutinin titers and changes in the salivary counts.

The data on salivary counts for the 13 unvaccinated controls is illustrated in Table III. Increases and decreases in average counts appeared in this group also, but all changes were found to be within the limits of chance variation. The average agglutinin titers for the group, (see *fig. 3*), show little variation during the period of study.

Clinical and X-ray examinations of the teeth of all persons were repeated at 3 and 6 month intervals after the first survey. There were no new carious lesions, nor any apparent changes in existing untreated lesions in either the vaccinated or control groups.

SUMMARY

1. Subcutaneous inoculation of human beings with both heat-killed and living vaccines, containing the strains of lactobacilli used in these tests, was accomplished 4 times at weekly intervals without inciting any severe local or general detrimental effects.

2. Vaccination increased the blood agglutinin titers for the strains used in the vaccines, the maximum being attained around 2 weeks after vaccination. The titers decreased after this period and were near the unvaccinated levels 5 months after the last injections. Controls showed no appreciable change in titer.

3. Salivary counts for lactobacilli over a period of 4 months demonstrated that 15 out of 20 persons (75%) in the immunized group had apparent reductions in average salivary counts. Statistical analysis indicated that only 5 out of the 20 (25%) could be regarded as having reductions greater than could be accounted for by chance. The average counts for lactobacilli in the controls showed changes which were within the limits of chance variation.

4. The average agglutinin titer levels were about the same for both sections of the immunized group, one with reductions in average counts within the

limits of chance variation. No relationships could be established between the blood agglutinins and the changes in average counts.

5. The results seem to indicate that the immunization, as performed in this study, had no consistent effect on the numbers of lactobacilli cultivable from the oral cavity.

REFERENCES TO LITERATURE

1. Canby, C. P., and Bernier, J. L., Bacteriologic and Immunologic Studies in Dental Caries: A Preliminary Report, *J. A. D. A.*, **29:** 606–617, April, 1942.
2. Kulp, W. L., and White, V., A Modified Media for Plating *Lactobacillus acidophilus*, *Science*, **76:** 17–18, July 1, 1932. As Modified by Hadley, F. P., *J. D. Res.*, **13:** 415–428, October, 1933.
3. Flosdorf, E. W., and Mudd, S., Improved Procedure and Apparatus for Preservation of Sera, Micro-Organisms and Other Substances: Cryochem Process., *J. Immunol*, **34:** 469, June 1938.
4. Hadley, F. P., Bunting, R. W., and Delves, E. A., Recognition of *Bacillus acidophilus* Associated with Dental Caries: A Preliminary Report, *J. A. D. A.*, **17:** 2041–2058, November, 1930.
5. Rider, P. R., *An Introduction to Modern Statistical Methods*, (John Wiley and Sons, Inc., 1939), p. 91–93.

A RELATIONSHIP BETWEEN DENTAL CARIES AND SALIVA[1]

NAOMI C. TURNER AND EDWARD M. CRANE

Chemical Laboratory, Radcliffe College, Cambridge, Mass.

This paper reports studies of variations in the rate of starch hydrolysis in the saliva of different individuals and shows a relationship between the rate of hydrolysis and the amount of caries. Several attempts have been made to find a significant difference in the chemical constitution of saliva associated with dental caries as opposed to the saliva of caries-free individuals (1). We report here a functional difference. In carrying out these studies on hydrolysis, the authors had before them no previous reports of investigations of this type.[2]

In the hydrolysis of starch, as indicated under standardized conditions by the spot-test disappearance of starch-iodine blue, saliva associated with severe caries acts rapidly. Individuals without caries produce saliva which hydrolyzes starch only slowly.

The test outlined below has been reduced to its simplest terms in order to study a sufficiently large number of cases without undue inconvenience to the patients and clinical assistants. Consequently, the experimental error is larger than it might be in a more elaborate test; however, it was found that the hydrolyzing time of one individual used as control could be consistently reproduced. The test is strictly empirical. The end point is not easily read, both because of the difficulty in distinguishing between deep blue and deep brown and because the spot test does not "keep" for comparison. Great care was exercised to work on an objective basis. We hope that the work will be checked by others.

After some experimenting the routine was established as follows: Saliva was taken not less than 2 hours after eating, the only stimulus being psychological and that of the explorer as the dental examiner charted the cavities. The sample was collected in a trap in the saliva ejector suction line. Each break in the enamel was recorded as a cavity; that is, each fissure, carious spot, and filled tooth counts 1 in the figure given for cavities. Saliva was tested beside the dental chair within 1 to 3 minutes of collection, since it was observed that the activity of saliva decreased notably on standing.

[1] Received for publication May 11, 1944. Revised by authors, October 2, 1944.

[2] Since the publication of our brief note in *Science* (2) a note from Dr. H. J. Florestano has called to our attention his interesting study along this line made in 1938 (3). Using the starch-iodine reaction as an index, he concluded from a study of 166 cases that there is a relationship between the amount of caries and the diastatic index. Differences in technique, particularly our use of very fresh saliva, appear to account for the wider difference and sharper results in our investigation. Earlier Hubbell (4) had made studies along this line in which she found no such relationship. (Gore [*J. D. Res.*, **21**: 383, 1942] has reported related studies, Ed.)

TABLE I

NUMBER OF INDIVIDUALS	CAVITIES	AVERAGES	HYDROLYZING TIME IN MINUTES	AVERAGES
	0		60	
	0		48	
	0		38	
	0		32	
	1		60	
	1		39	
	2		30	
	3		35	
	3		28	
10	3	1.3	27	40
	4		20	
	4		19	
	4		18	
	5		20	
	5		19	
	6		26	
	6		16	
8	6	5.0	10	18.5
	7		18	
	7		6	
	8		11	
	8		11	
	8		7	
	8		7	
	8		5	
	8		5	
	8		3	
	9		15	
	9		14	
	9		9	
	9		6	
14	9	7.6	5	8.7
	10		9	
	10		9	
	10		8	
	10		8	
	10		7	
	10		7	
	10		7	
	10		6	
	10		4	
	11		8	
	11		7	
	11		2	
13	12	10.4	7	6.8
	13		3	
2	14	13.5	5	4.0
	20		2	
2	21	20.5	1.5	1.8
	32		0.5	
2	33	32.5	1.5	1.0

At room temperature (23°C.) 1 cc. of saliva was mixed with 1 cc. of 1% starch solution and the stop watch was started. On a spot plate 4 drops of the mixture was stirred with 1 drop potassium triiodide solution (5g I_2, 10g KI, 80 cc. water) at the following intervals: 30 seconds, 1, 1½, and 2 minutes; after 2 minutes, 1 minute intervals were used up to 10 minutes, after which 5 minute intervals were used until there was an indication that the end point was near, when

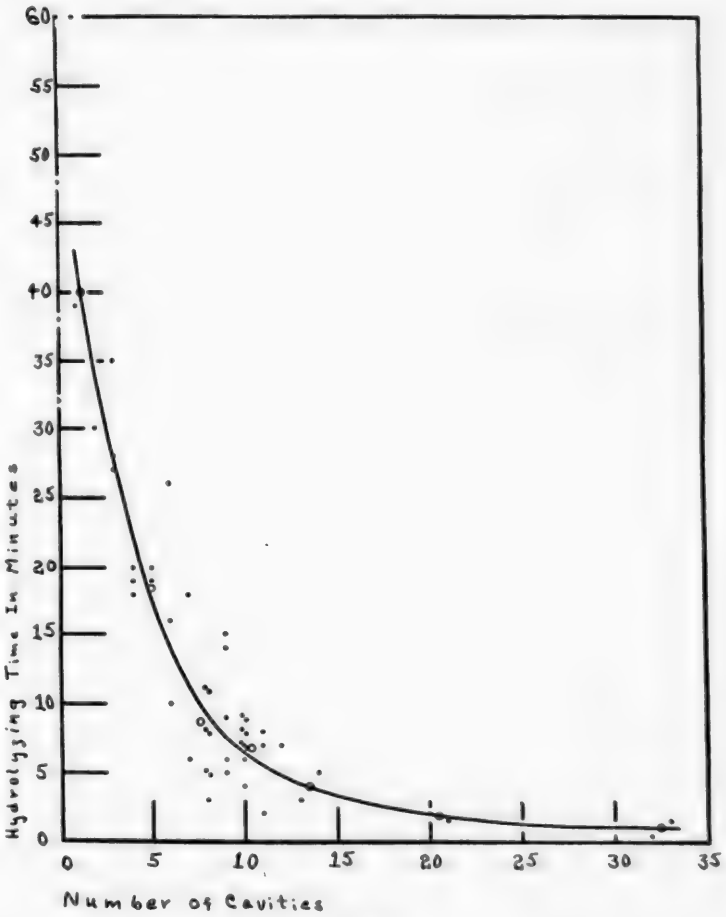

Fig. 1

minute intervals were resumed. The disappearance of blue-purple color was called the end point. The spot appeared brown. No end point was accepted unless 2 successive spots agreed in color, and unless the brown color persisted on high dilution.

Salivas were tested with "Hydrion" paper, and although there was considerable variation in pH, there was no correlation between these readings for pH and the amount of caries.

Temperature as well as the concentration and proportion of the reagents is a critical factor in reading hydrolyzing time. Diluted potassium triiodide solution gives a blue spot where the more concentrated indicator shows brown. Consequently the times given below cannot be taken as those of complete hydrolysis, but they do represent the attainment of the same degree of hydrolysis in each case. It is also necessary to observe the color of the spot immediately on stirring, for on standing it may revert to blue. That is an extraneous circumstance as shown by the fact that a solution of pure dextrins gives, with potassium triiodide, a brown spot which reverts to blue-purple on standing.

Table I shows the results of testing 51 individuals; the age range was 5 to 20 years though the preferred age was 8 to 10 years. *Fig. 1* shows the same data, experimental points being indicated by dots, average values by circles.

The authors wish to acknowledge with gratitude the assistance of Dr. P. R. Howe, Miss Rosalie K. Kirby, and others of the staff of the Forsyth Dental Infirmary.

REFERENCES TO THE LITERATURE

1. Becks, H., Wainwright, W. W., and Young, D., *J. D. Res.*, **20:** 171, 1941; Marshall, *Am. J. Physiol.*, **43:** 212, 1917; Karshan, M., Krasnow, F., and Krejci, L. E., *J. D. Res.*, **11:** 573, 1931.
2. Turner, N. C., and Crane, E. M., *Science*, **99:** 262, 1944.
3. Florestano, H. J., Faber, J. E., and James, L. H., *J. A. D. A.*, **28:** 1799, 1941.
4. Hubbell, R. B., *Am. J. Physiol.*, **105:** 436, 1933.

DENTAL CARIES IN THE COTTON RAT[1]

II. Production and Description of the Carious Lesions

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An experimental animal which is readily susceptible to dental caries has long been recognized to be the prerequisite for research in the causes and control of dental caries. Shaw, Schweigert, McIntire, Elvehjem and Phillips (1) have given evidence that the cotton rat (*Sigmondon hispidus hispidus*) is much more susceptible to dental caries than any other experimental animal previously reported. The evidence presented indicates the opening of a fertile field for dental research. The method of production and the description of the carious lesions in the cotton rat are to be reported here.

EXPERIMENTAL

The cotton rats used in these experiments were obtained from our stock colony, which was maintained originally on the Steenbock stock ration No. 14 which consisted of: ground corn 71.5%, linseed oil meal 15%, crude casein 5%, alfalfa leaf meal 2%, butter fat 5%, $CaHPO_4$ 1%, and NaCl (iodized) 0.5%. This ration was supplemented with greens. When the rate of reproduction was found to be unsatisfactory on this dietary regimen, the stock colony was fed a ration which consisted of: Ideal dog food (VT) 71.5%, coarsely ground corn 11%, crude casein 7%, alfalfa leaf meal 7%, and corn oil 3.5%. This ration was supplemented with greens and it promoted satisfactory reproduction. No differences were observed in the growth rate and development of dental caries in the young cotton rats from the change in stock ration.

The young were weaned at the age of 3 weeks, and weighed 20 to 25 gm. Then they were placed in individual cages on raised screens.

The basal caries-producing ration (801) consisted of Labco casein 18%, sucrose 73%, salts IV (2) 4%, and corn oil 5%. To each 100 gm. of ration was added: thiamine 250 ug., riboflavin 300 ug., nicotinic acid 2.5 mg., pyridoxine 250 ug., pantothenic acid 2 mg., choline 100 mg., inositol 100 mg., and p-aminobenzoic acid 30 mg. One drop of haliver oil was fed to each rat weekly. A modification (802) of this basal ration was found to give more uniform growth and has, therefore, been used recently as the caries-producing ration. It consisted of an increase in the casein to 24%, at the expense of the sucrose.

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In the preliminary experiments the cotton rats were left on experiment for 10 to 14 weeks. It was found that 14 weeks was the optimum experimental period for accurate evaluation of the number of carious lesions and the extent of each individual lesion. At that time, the lesions were large enough to be readily detected, and yet not so large that any extensive fracturing of the cusps had occurred. A minimum amount of fracturing was a prime necessity, since widespread fractured areas would involve and obscure several of the original carious lesions.

The caries-preventing rations were: coarse and fine stock ration No. 14, ration 801 with the sucrose replaced by coarse or fine dextrin, and the dog food stock ration.

At the end of the experimental period, the animals were sacrificed by decapitation. The heads were immediately fixed in 95% ethanol, and left there for 24 to 48 hours. The jaws were then prepared for grinding by dissecting off the skin and flesh. The jaws of each head were tied together and stored in fresh ethanol until observations could be made.

The jaws were never allowed to dry, and were never boiled in order to ease the process of cleaning. It was found that drying or boiling greatly obscured the softening changes which were present in the carious lesions prior to fixation. Frequently we found that such drastic treatment caused artefacts such as drying and shrinking of the carious material, and/or crumbling and sloughing of the affected areas.

By leaving the heads in 95% ethanol for 24 to 48 hours, it was found that all the soft tissue was hardened sufficiently to be easily stripped from the bones. By this type of treatment all jaws were fixed in such a way that, if desired in view of findings in the grinding and observation process, they could be used for histological examination of the more minute structural details.

The observations on the molar teeth reported here were made by grinding and observing several successive planes parallel to the occlusal surface. After each grinding step, the newly exposed plane was observed under reflected light at a magnification of 30 diameters with a binocular dissecting microscope. Each lesion observed was carefully explored with a No. 42 fine, smooth, dental brooch to determine the depth and width of the softening area as a supplement to the visual changes in structure, color, and opalescence. When the entire vertical axis of a lesion had been ground through, a composite picture of the lesion was derived by the summation of the portions observed in the successive planes. Such a composite picture was essential in order for the observer to accurately evaluate the extent of the lesion.

In this paper, only the observations of the carious lesions made by the alternate grinding and observation procedure will be reported. Histological observations will be reported later.

RESULTS

Description of the Dentition of the Cotton Rat: The dentition of the cotton rat is similar to that of the white rat: monophyodont, 1 incisor and 3 molars

in each quadrant of the jaws, continuously erupting incisors, and molars which are more highly developed than the incisors and which are limited in development to the early period of life. The molars are similar to those of the white rat in most respects. The chief difference, peculiar to the species, which may be the fundamental difference in so far as caries susceptibility is concerned, is the extremely narrow, deep, occlusal fissures in the molars of the cotton rat.

When the molars of the cotton rat were erupted, there were definite, distinct cusps as in the white rat. However, in the cotton rat, the completely transverse occlusal fissures separating the cusps were very shallow. A very small region of prenatally enamel-free dentin was usually present in each cusp which became larger as the cusp was more deeply abraded. Since the enamel was more resistant, the enamel rim was perceptibly higher than the exposed dentin. After a period of 3 or 4 weeks of occlusal contact, enough attrition has occurred that the cusps have been abraded to a level below the bottom of the original shallow, transverse, occlusal fissures which separated the individual cusps. In this way the original, distinctly individual cusps became connected at the occlusal surface. Thus, the exposed dentin region of a cusp became connected to the similar area in the next cusp mesial or distal to it by a narrow strip of exposed dentin, flanked on each side by a slightly higher rim of enamel. Thus, in the cotton rat, the occlusal surface of the molar, shortly after its eruption, had a continuous region of exposed dentin extending from the mesial to the distal cusp.

The occlusal fissures which lie between these connecting cusps were exceedingly narrow and deep. They extended from either the labial or lingual surface $\frac{1}{3}$ to $\frac{2}{3}$ across the molar, and from the occlusal surface almost to the cemento-enamel junction.

Regions of Tooth Decay: Tooth decay in the cotton rat occurred, almost exclusively, deep in the occlusal fissures of the molars. A very low incidence of decay was observed on the proximal surfaces and on the exposed occlusal surfaces. The rarest location where decay was observed was along the gingival margin. The progress of the carious lesions in all 4 regions appeared to be identical. No lesions were observed in the incisors.

The carious lesions in the occlusal fissures began at such a depth that early lesions were not evident upon inspection of the intact tooth. When decay had progressed considerably, fracture of portions of the undermined cusp would occur. Only then would the lesion become evident from the occlusal surface. Since it was desirable to study the teeth before any extensive amount of fracturing had occurred to obscure the original picture of the processes of decay, an alternate grinding and observation procedure similar to that of Cox and Dixon (3) was necessary to expose the early carious lesions hidden deep in the occlusal fissures.

The first indication of decay varied. In most cases the degree of darkening of the enamel varied from a slight yellow tinge to a definite brown. This was accompanied by a disappearance of the pearly opalescence and the striae of the enamel. Even at this early stage, there was a definite softening of the

enamel which became obvious when the suspected lesion was probed with a No. 42 fine, smooth, dental broach. At this time, the softening was accompanied by a definite resilience which was soon lost as the carious lesion progressed.

The destruction of the enamel appeared to be quite rapid once the initial lesion was established. Wide areas of enamel had been invaded before much penetration of the dentin occurred. The lesion then spread very rapidly into the dentin until large areas of the cusp were involved. Once the early stages of the process have passed, there was usually no color to be observed in the

TABLE I

Typical observation record of cotton rat fed ration 802 for 14 weeks

JAW	LEFT		RIGHT		TOTAL	
	Position	Extent	Position	Extent	No. of caries	Extent
Upper	7	2+	8	2+		
	9	3+	10	2+		
	11	2+	12	3+		
	13	2+	14	2+		
	15	3+	16	2+		
Total.....	5	12+	5	11+	10	23+
Lower	23	2+	24	2+		
	25	2+	26	3+		
	29	3+	30	2+		
	31	4+	32	4+		
	33	4+	34	3+		
	35	2+	36	2+		
	37	3+	38	3+		
	39	4+	40	4+		
Total.....	8	24+	8	23+	16	47+
Grand total.....	13	36+	13	34+	26	70+

lesions, other than a very faint yellow replacing the pearly white opalescence of the enamel and the faint pink of the dentin.

About this time fracture began to occur. Usually no large portions of the undermined cusp broke away at any one time; but instead small chips were broken off by the pressure of mastication. This was a progressive development occurring as the cusp was more deeply undermined by the carious lesions. Finally fracture progressed until the entire cusp had been lost. Where a cusp had been undermined by a carious lesion in the fissure on each side, fracture of the cusp could, and frequently did, occur from both sides. This eventually would result in the total loss of the cusp. This would make it impossible to evaluate the carious processes of that animal as accurately as if it had been killed before fracture had become so extensive.

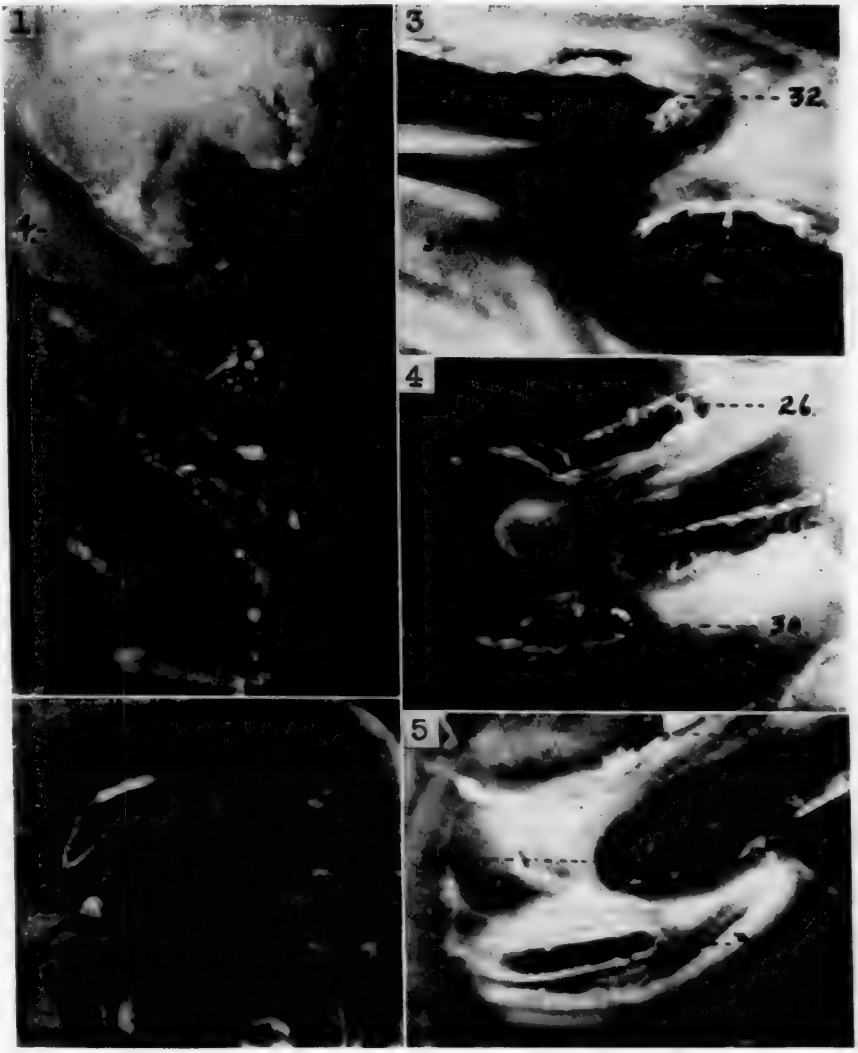

Fig. 1. Occlusal surface of an upper right first molar. No carious lesions are visible from surface. However, upon grinding and observation, it was found that there was 2+ lesion in fissure 6 and an early 3+ in fissure 8. ×40.

Fig. 2. Transverse plane of lower right third molar. Note normal enamel surfaces along both sides of fissures 38 and 40. ×40.

Fig. 3. Transverse plane of lower right second molar. Note 1+ carious lesion on mesial surface of fissure 32. Definite darkening of enamel can be seen and upon probing this lesion, enamel was found to be already quite definitely softened. ×100.

Fig. 4. Transverse plane of lower right first molar. Note 2+ carious lesion on distal surface of fissure 26. There is wide area of enamel involved with very little penetration of dentin visible yet. In fissure 28, an early 4+ lesion is visible on distal surface. ×40.

Fig. 5. Transverse plane of lower right first molar. Note 3+ carious lesion involving mesial surface of fissure 30. There is wide area of enamel and dentin penetration without any fracturing of cusp. 4+ lesion is present on distal surface of fissure 26. ×40.

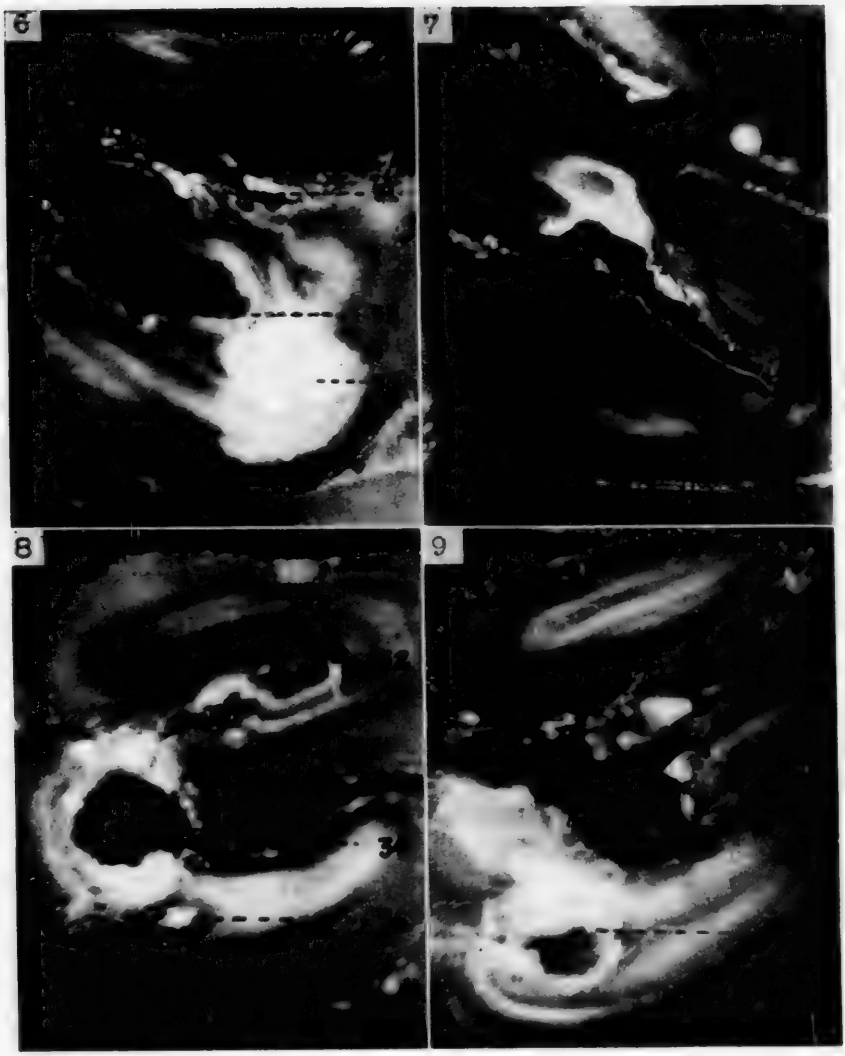

Fig. 6. Transverse plane of lower left second molar. Note early 4+ carious lesion on distal surface of fissure 33 where there has been slight fracturing of enamel along wide area. In this tooth there is also 2+ lesion on distal surface of fissure 31 and 3+ lesion involving most of bottom of fissure 35. ×40.

Fig. 7. Transverse plane of upper right first molar. Note late 4+ carious lesion on mesial surface of fissure 8. Considerable fracturing has occurred and lesion has progressed deep into dentin. ×40.

Fig. 8. Transverse plane of lower right second molar. Note early 5+ carious lesion at lingual end of fissure 34. Large amount of fracturing has occurred and lesion is just about to break through into fissures 32 and 36. ×40.

Fig. 9. Transverse plane of lower right second molar. Note late 5+ carious lesion involving fissures 32 and 34. At this stage accurate evaluation is impossible since this lesion may have been due to one lesion in either fissure or lesion in each fissure. There is also an early 4+ lesion in fissure 36. ×40.

TABLE II

Comparison of caries-producing ability of various rations when fed to the cotton rat

RATION	NUMBER OF WEEKS	NUMBER OF ANIMALS	AVERAGE NUMBER OF CARIOUS LESIONS (D)	AVERAGE EXTENT OF CARIOUS LESIONS (E)	AVERAGE EXTENT OF EACH CARIOUS LESION (E/D)
801	10	9	12.3	26.7+	2.2+
	12	4	20.3	47.8+	2.4+
	14	24	25.5	70.7+	2.8+
802	12	5	21.8	41.2+	1.9+
	14	26	26.9	72.2+	2.7+
Coarse dextrin	12	3	2.0	2.0+	1.0+
	14	5	2.4	6.6+	2.8+
Fine dextrin	12	1	1	1.0+	1.0+
Coarse stock ration No. 14	12	3	6.0	12.0+	2.0+
	14	4	6.8	15.5+	2.3+
	50	23	3.0	7.6+	2.5+
Fine stock ration No. 14	12	3	6.0	8.0+	1.3+
Dog food ration	14	3	5.3	10.3+	2.0+

TABLE III

Relative incidence and extent of carious lesions in molars of cotton rats fed caries-producing rations for 14 weeks

RATION	NO. OF COTTON RATS (N)	JAW	MOLAR	TOTAL NUMBER OF CARIOUS LESIONS (A)	TOTAL EXTENT OF CARIOUS LESIONS (B)	AVERAGE EXTENT OF EACH CARIOUS LESION (B/A)	NUMBER OF CARIOUS LESIONS PER MOLAR (A/2N)	NUMBER OF SUSCEPTIBLE REGIONS PER MOLAR (C)	PER CENT OF SUSCEPTIBLE REGIONS THAT DEVELOPED CARIOUS LESIONS (A/2N × 1, C × 100)
801	24	Upper	I	68	122+	1.8+	1.4	4	35
			II	123	398+	3.0+	2.6	3	86
			III	84	196+	2.3+	1.8	3	58
			Total	275	716+	2.2+	5.8	10	58
		Lower	I	117	280+	2.4+	2.4	5	49
			II	127	407+	3.2+	2.7	3	89
			III	92	302+	3.3+	1.9	2	96
			Total	336	989+	2.9+	7.0	10	70
802	26	Upper	I	72	129+	1.8+	1.4	4	35
			II	137	372+	2.7+	2.6	3	88
			III	94	231+	2.5+	1.8	3	60
			Total	303	732+	2.4+	5.8	10	58
		Lower	I	158	378+	2.4+	3.0	5	60
			II	143	437+	3.1+	2.8	3	92
			III	99	336+	3.4+	1.9	2	95
			Total	400	1151+	2.9+	7.7	10	77

The surface exposed by the fracture did not appear to be attacked by the processes of decay at any greater rate than if fracture had not occurred. Instead decay progressed into the exposed face from the original lesion at about the same rate as if no fracture had occurred to expose further regions of the tooth to decay. Fracture of a different type occurred occasionally in those cotton rats which were fed coarse stock rations. The fractures in these cases were not due to an undermining of the cusp by a carious lesion, but were caused by the mastication of coarse food particles which weakened the tooth structure. Here again after this type of fracture, the newly exposed area of the molar did not appear to be susceptible to tooth decay. It was only rarely that a carious lesion was observed on this exposed surface.

The method of recording the position of the carious lesions and the arbitrary system for the evaluation of the extent to which the decay had progressed were presented in a previous paper (1). The record of the observations of a typical cotton rat fed ration 802 for 14 weeks is given in Table I. *Figs. 1* to *9* present examples of normal teeth and various stages of the carious lesions.

Production of Carious Lesions: The caries-producing ability of various rations is presented in Table II. The high sucrose rations, 801 and 802, when fed for a period of 10 to 14 weeks, resulted in a very high incidence of carious lesions in the occlusal fissures of the cotton rat. The optimum experimental period was found to be 14 weeks. The high incidence of carious lesions, observed in the cotton rats fed the sucrose rations, is in great contrast to the very low incidence in those fed the dextrin rations or the stock rations. In this table it will be seen that the extent of each carious lesion, at the end of the experimental period, is almost the same regardless of whether that lesion was developed in an animal on a sucrose ration or on one of the caries-preventing rations. This may indicate that the carious lesions once begun, progress at a speed relatively independent of the ration which the animal was fed. However, in the cotton rat the process of the initiation of the lesion was very definitely dependent on the ration.

In order to indicate the reliability of the two sucrose rations in producing caries in the cotton rat, comparative analysis of the incidence of dental caries in the individual molars of all animals fed both rations is presented in Table III. An almost identical incidence and extent of the lesions can be seen in respective molars in the groups fed the two rations.

DISCUSSION

The cotton rat fed sucrose ration 801 or 802, is more susceptible to tooth decay than any other experimental animal yet reported. This is in contrast to the low incidence on a similar ration in which sucrose is replaced by dextrin or on rations composed of natural foodstuffs. The topographical structure of the cotton rat molar probably has a very important relation to the incidence of caries on a sucrose ration. The deep narrow fissures of the cotton rat molar are in great contrast to the wide shallow fissures of the white rat molar where the impaction of a large fermentable particle appears to be a prerequisite for caries production. Thus, in the cotton rat the factor of particle size (which is

so difficult to control in the white rat) has been eliminated by the use of a synthetic type of ration containing a high level of sucrose. The high, and consistent rate of incidence of carious lesions in the cotton rat makes possible a thorough study of the causes of dental caries and their prevention.

The cotton rat is far removed phylogenetically from the human species. It would be desirable to have a highly susceptible species that was much more closely related to the human. However, until that animal is found, the cotton rat can be profitably used for many types of dental research. The optimum experimental period of 14 weeks is sufficiently short that results can be obtained and rechecked several times in the period which would be necessary in the monkey or the human.

Under our experimental conditions, the necessity for an experimental period of 14 weeks must be emphasized. The progress of decay is very rapid in the cotton rat. Most of the lesions are initiated before 10 weeks on experiment, and are reasonably visible at 10 and 12 weeks. However, the optimum time for observation is after 14 weeks on experiment. At that time, the lesions are large enough to be readily observed by our methods of investigation. However, any extension of the experimental period beyond 14 weeks to allow the lesions to become larger is actually futile, since fracturing of the undermined cusps after that time has become so great that accurate evaluation is no longer possible. The number of carious lesions, though each lesion individually is larger, is reduced by the merging of lesions and their fractures, each original lesion having lost its individuality.

As yet only one strain of cotton rat has been studied to determine its susceptibility to caries formation. Other strains are now being investigated and additional ones will be studied as they become available.

SUMMARY

The type of carious lesions occurring in the molar teeth of a strain of cotton rats and the method of production of these carious lesions is presented. The sucrose rations which were used to produce the high incidence of carious lesions are described, along with the experimental handling of the cotton rats. The high susceptibility of the molar teeth of the cotton rats fed a sucrose ration is in very great contrast to the high resistance of those fed the various caries-preventing rations.

The authors believe that the cotton rat is the most caries-susceptible experimental animal yet reported. Its use is recommended in many types of research involving the causes and control of dental caries.

We are indebted to Merck and Co., Rahway, New Jersey for the synthetic vitamins and to Abbott Laboratories, North Chicago, Illinois for halibut liver oil.

The authors wish to express their appreciation to Anna Lou Hansen for technical assistance.

REFERENCES TO LITERATURE

1. Shaw, James H., Schweigert, B. S., McIntire, J. M., Elvehjem, C. A., and Phillips, Paul H., *J. Nutrition*, **28**: 333, 1944.
2. Phillips, Paul H., and Hart, E. B., *J. Biol. Chem.*, **109**: 657, 1935.
3. Cox, Gerald J., and Dixon, Sara F., *J. D. Res.*, **18**: 153, 1939.

A PRELIMINARY SURVEY OF THE POUCHES AND DENTITION OF THE SYRIAN HAMSTER[1]

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In recent years, the Syrian or golden hamster (*Cricetus auratus*[2]) has become increasingly useful as a laboratory animal. Its potential value in dental research was suggested by Arnold (1) in 1942. Although the albino rat has been used extensively for dental investigations, it has not been entirely satisfactory because certain features of its dentition are not analogous to the human (2). Preliminary observations of experimentally produced dental lesions in the molar teeth of the hamster (1, 3) have been encouraging. The possibility that this animal may prove useful for dental studies and eliminate certain objectionable features of the rat has prompted a preliminary survey of some general characteristics of its oral cavity.

THE BUCCAL POUCHES

The presence of 2 well-developed buccal pouches (*fig. 1*) is a distinctive feature of hamsters. They are balloon-like structures located beneath the dermis along the lateral side of the head and neck (*fig. 2*) and consist largely of dense fibrous elastic tissue. Each is suspended by a thin muscle slip which inserts into the lumbar fascia in the mid-dorsal line. The pouches open into the mouth through the diastema between the incisors and the molars. Apparently they have no digestive function and are not related to the salivary glands.

The pouches are employed in carrying food. The extent to which they are used in the laboratory depends on the nature of the diet, and to some degree, on the sex of the animal. Hamsters on a finely milled diet seldom fill them, but those receiving whole corn or food pellets habitually carry their ration from the

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[2] The golden hamster, first described by Waterhouse in 1839 (4, 5) is native to Syria and is smaller than common European species. It is covered by smooth short fur, golden-brown dorsally and light gray ventrally. Females are consistently larger and heavier than males, but rarely exceed 7 inches in length or 200 grams in weight (6). These animals become sexually mature when 7 or 8 weeks old and produce litters of 6 or 7 young after a gestation period of 16 days. Few females have more than 4 litters; nevertheless, they continue to ovulate every 4 days until quite old (7). A winter breeding pause from October to February may be partially overcome by providing animal quarters with more light. Treatment with gonadotropic hormones permitted pregnancies in normal animals during the breeding pause and in sterile and senile females during the natural breeding season (8).

food container to a rear corner of the cage. Females show more inclination to store food than males, but under normal conditions, neither retains food in the pouches for long periods of time. Very young animals tend to keep food in their pouches, but the tendency disappears with maturity.

Anatomically and physiologically the pouches do not seem to be related to the teeth. However, possible effects of retained food particles on the oral flora and on the chemical properties of the saliva should not be overlooked. No information regarding the relationship of the pouches to conditions affecting the dentition is available.

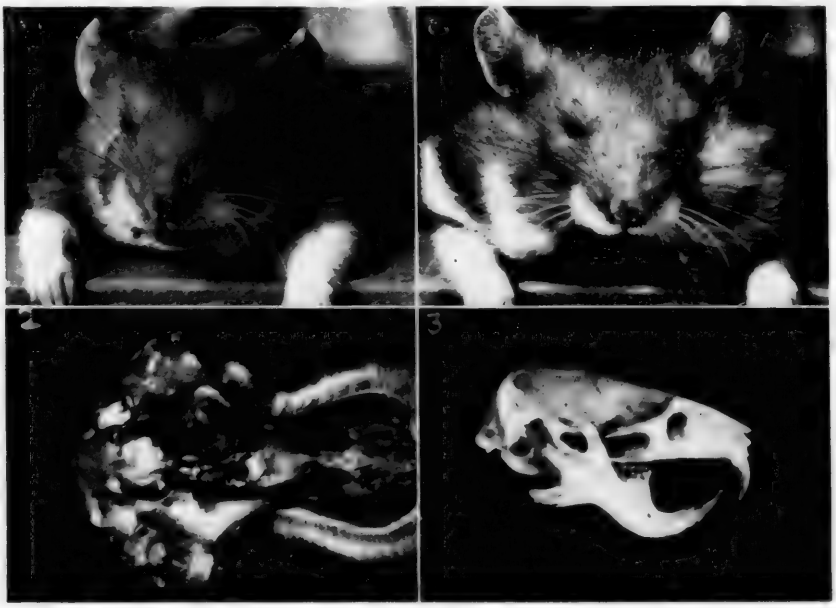

Fig. 1. Head of Syrian hamster: A. Pouches empty; B. Pouches filled with food.

Fig. 2. Dissection, dorsal view. Pouches are filled with corn and have been drawn away from head to show suspensory muscle.

Fig. 3. Skull of mature Syrian hamster showing notched mandibular incisor and typical occlusion of molar teeth. Projection found in rat on lateral aspect of ramus beneath sigmoid notch is absent in hamster.

THE TEETH AND THEIR ERUPTION

The dentition of the Syrian hamster is monophyodont, polybunodont, and brachyodont. The dental formula is typical of rodents:

$$I\,\frac{1}{1}\quad C\,\frac{0}{0}\quad PM\,\frac{0}{0}\quad M\,\frac{3}{3}$$

Primary eruption of teeth in the hamster, as studied by gross examination, appears to be quite regular and subject to a normal range of variation. The process may be slightly more advanced in rapidly developing animals and small litters than in slowly growing animals and large litters.

Intraoral eruption begins very soon after birth and extends over a period of approximately 40 to 45 days. The incisor teeth are the first to appear. They usually erupt within 24 hours after birth and attain occlusion within one day. Attrition of the incisal tips begins immediately.

Incipient calcification of cusp tips in first molars may be seen in radiographs of 2-day old animals. These teeth become evident beneath the gingiva in animals 5 or 6 days of age. Extragingival eruption starts on the seventh or eighth day and is slightly more advanced in the mandible than in the maxilla. Functional occlusion is attained in about 1 day; as these teeth erupt, the hamster begins to eat solid food.

Early calcification of the second molars is evident in radiographs of 7-day old animals. These teeth generally begin to erupt when the animal is 12 to 13 days old, appear first in the mandible, follow in the maxilla about 24 hours later, and reach occlusion in 3 or 4 days.

Radiographic evidence of cusp calcification in third molars may be found in 18-day old animals. Eruption is comparatively irregular and slow, beginning in the mandible about 30 days after birth and in the maxilla two or three days later. The teeth do not appear to come into occlusion until 40 to 45 days after birth.

THE INCISORS

The incisor teeth exhibit typical rodent characteristics. These teeth grow, calcify, and erupt continuously during the life of the animal, are separated from the molars by a diastema greater in the maxilla than in the mandible (*fig. 3*), and consist primarily of dentin, covered by enamel labially and by cementum laterally and lingually. They increase in cross section and in the length of the extra-alveolar segment with the growth of the animal. Radiographs of the heads of new-born animals sacrificed over a 5-week period reveal rapid development and calcification of the skull, incisors, and molars (*fig. 4*). Average measurements of the extra-gingival lengths of hamster incisors are indicated in Table II.

The rate of incisor growth varies considerably. Further investigation will probably reveal not only irregularity between similar animals but differences due to age, sex, diet, and season. Measurements in 10 mature males on a basic ration (Purina rabbit chow) over a 4-week period reveal average eruption rates of approximately 2.5 plus or minus 0.5 mm. per week in the maxilla, and 4.0 plus or minus 0.7 mm. per week in the mandible. As maxillary incisors are short and attrition is rapid, the extragingival portion may replace itself within a one week period. The mandibular incisors, having a greater extragingival length, require about 2½ to three weeks for replacement.

The maxillary incisors grow from crypts in the anterior part of the maxilla and erupt through the premaxilla. They are symmetrical segments of a 2-dimensional spiral increasing in size as the animal matures. The labio-lingual dimension of these teeth is somewhat smaller and the mesio-distal width slightly greater than in mandibular incisors. All dimensions are greater in females than in males (Table III). The incisal edges of maxillary incisor teeth are

sharply beveled by attrition; the worn surfaces are short and curve lingualward to the gingival margin.

Fig. 4. Radiographs of heads of hamsters 1, 7, 14, 21, and 35 days old showing rapid development of calcified structures.

TABLE I

Ages at which teeth erupt in the Syrian hamster

	RADIOGRAPHIC EVIDENCE OF CALCIFICATION	ERUPTION BEGINS	OCCLUSION ATTAINED
	days		*days*
Incisors		~24 hrs	2
1st Molars	2	7–8 days	8–9
2nd Molars	7	13 14 days 12–13 days	16–17
3rd Molars	18	32 35 days 30–32 days	40–45

The mandibular incisors grow from odontogenic tissues completely enclosed within the ramus of the mandible, pass through the entire length of the body, and erupt near the symphysis (*fig. 3*). These teeth are segments of a 3-dimensional spiral. The radius of curvature is greater than that of the maxillary

incisors, and increases with the animal's growth. The incisal bevel is concave, longer than in maxillary incisors, and extends lingualward to the gingival margin, where it ends abruptly in a notch.

Pigmentation of the incisors, first evident in maxillary teeth about 18 days after birth and in mandibular incisors several days later, becomes pronounced in 30-day animals. Normally it ranges from pale yellow to yellow-orange and increases in intensity with age. Maxillary teeth, as a rule, are more deeply colored than mandibular, but considerable variation occurs in animals alike in all general respects. Occasionally no difference in pigmentation can be detected between upper and lower teeth. Either may appear devoid of pigment independently or simultaneously. One incisor may be pigmented and the adjacent tooth non-pigmented while the same condition is reversed in the opposite jaw.

TABLE II

Approximate lengths of extragingival segments of the incisor teeth of hamsters 1 to 5 weeks old

AGE	MAXILLARY INCISORS	MANDIBULAR INCISORS
wks.	*mm.*	*mm.*
1	1.0	2.0
2	1.7	2.9
3	2.1	3.5
4	2.7	4.3
5	2.9	4.5

TABLE III

Average dimensions of gingival cross sections of the incisor teeth of 10 male and 10 female hamsters 165 days old

SEX	MAXILLARY INCISORS		MANDIBULAR INCISORS	
	Labio-lingual	Mesio-distal	Labio-lingual	Mesio-distal
	mm.	*mm.*	*mm.*	*mm.*
Male	1.58 ±0.07	1.42 ±0.04	1.74 ±0.07	1.33 ±0.05
Female	1.70 ±0.08	1.53 ±0.07	1.90 ±0.08	1.44 ±0.05

THE MOLARS

The basic pattern of hamster molar crowns (*figs. 5* and *6*) is a primitive one similar to that of molar forms found in the prehistoric multituberculate, Bolodon (9). This design,[3] characterized by 3 pairs of cusps in antero-posterior alignment, is found in maxillary and mandibular first molars but is modified in the second and third molars. The cusps are conical, sharply pointed, and separated by broad bucco-lingual sulci and deep occlusal fossae and grooves. They are

[3] Cusp arrangement of the molars in situ stimulates the appearance of several human maxillary bicuspids in alignment. Each pair of cusps is slightly rotated so that the buccal cusps are more distal in position than the lingual. The arrangement permits lateral excursions of the mandible with interdigitation.

joined by a system of bucco-lingual and mesio-distal ridges which form H-patterns on the occlusal surfaces. The bucco-lingual ridges form mesial and distal walls of the occlusal fossae; mesio-distal ridges connect approximating bucco-lingual ridges and divide the sulci into buccal and lingual portions.

To facilitate description of the molars, the following nomenclature is employed:

Paracusps, minor mesial cusps: smallest cusps on the crown mesially located beside the mesocusps; generally paired.

Mesocusps, mesial cusps: prominent paired cusps mesially located on each crown.

Distocusps, distal cusps: paired cusps distally located on each crown.

The maxillary molars (*fig. 7*) are characterized by several distinct features. The basic pattern of the first molar is modified in the second and third by a marked reduction in size of the buccal paracusp, loss of the lingual paracusp,

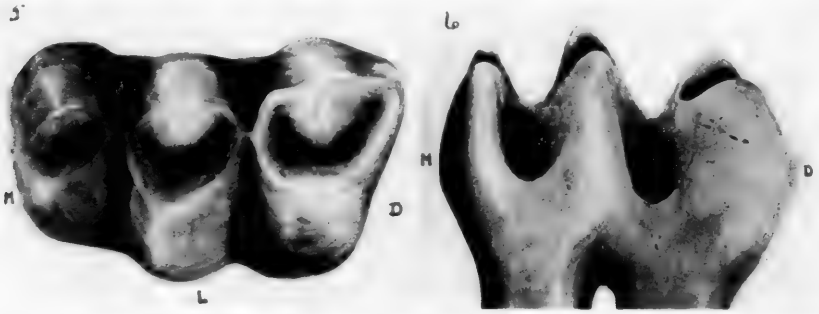

Fig. 5. Occlusal view of newly erupted maxillary 1st molar illustrating the basic design of hamster molar teeth. Note H patterns formed by system of intercuspal ridges. Buccal cusps are slightly distal to corresponding lingual cusps. M—mesial; D—distal; L—lingual.

Fig. 6. Buccal aspect of newly erupted mandibular 1st molar further illustrating basic pattern of hamster molar teeth. Cusp pairs (mesial to distal): paracusps, mesocusps, distocusps. Prominent ridges connecting cusp pairs correspond to connecting bars in H designs. Lingual cusps are slightly more elevated than corresponding buccal cusps, a characteristic of mandibular molars. M—mesial; D—distal.

and a slight increase in size of the mesocusps. Although the lingual paracusp is absent on the second and third molars, these teeth have a prominent mesial convexity which is overlapped by the distal surfaces of adjacent teeth. Buccal cusps are higher than lingual. From a buccal or lingual aspect, axes of the buccal cusps are essentially parallel to the long axes of the teeth, whereas axes of the lingual cusps are deviated slightly distalward. The buccal sulci are deep, relatively short, and somewhat triangular in shape. They are terminated gingivally by a distinct ledge, or small tubercles found only on second or third molars between mesocusps and distocusps. The lingual sulci are more broad and long than the buccal and slope gradually to the gingival margin, where they are terminated by small ridges. Long axes of the teeth incline buccally and very slightly to the distal. Measurements of the molar crowns are given in Table IV.

The maxillary first molar has 6 cusps in the basic molar pattern. Paracusps are small and separated by a very minute pit fossa. Mesocusps are larger and separated by a deep oval fossa. Distocusps are the largest on the crown and are separated by a deep semicircular groove curving into the disto-buccal corner of the crown. The occlusal surface is marked by 2 typical H-form ridges, one between paracusps and mesocusps, the other between mesocusps and distocusps. Sulci are terminated gingivally by small ridges.

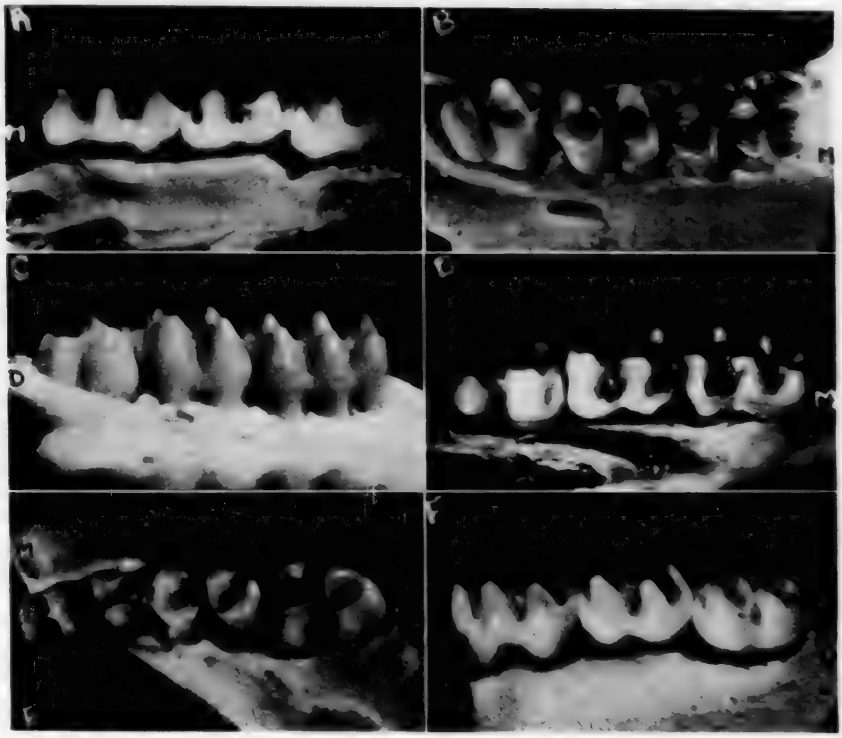

Fig. 7. Maxillary molars (A, B, C): A. Buccal view. Note reduction in molar size and paracusps from 1st to 3rd molar. Small tubercles on 2nd molar are poorly defined on 3rd. B. Occlusal view. Note increased bucco-lingual width of mesocusps in 2nd and 3rd molars C. Lingual view. Buccal cusps are higher than lingual. Note broad lingual sulci, overlapping contacts, and absence of lingual paracusps in 2nd and 3rd molars.

Mandibular molars (D, E, F): D. Buccal view. Lingual cusps are higher than buccal. Note reduction of paracusps in 2nd and 3rd molars. E. Bucco-occlusal view. Mesocusps are increased in bucco-lingual width in 2nd and 3rd molars. Note distal position of buccal cusps, and deeply invaginated buccal sulci. F. Lingual view. Cusps are inclined somewhat mesialward. Lingual paracusps are greatly reduced but not eliminated in 2nd and 3rd molars. M = mesial; D = distal.

more prominent buccally than lingually. The distal surface is broad and flat and overlaps the approximating surface of the second molar. The tooth has 4 roots whose lengths are about 3 times the height of the crown: mesial, buccal, and 2 lingual.

The maxillary second molar has 5 cusps and represents a modification of the basic design in which the buccal paracusp is markedly reduced and the lingual paracusp is absent. The buccal paracusp is separated from the mesocusps by a small shallow sulcus. The meso

cusps are slightly larger than any of the first molar cusps and are separated by a deep oval fossa. The distocusps, about equal in size to those of the first molar, are separated by a deep semi-circular groove which curves into the disto-buccal corner of the crown and accentuates the distal marginal ridge. The occlusal surface is marked by one H-ridge between the mesocusps and distocusps. The depth of the buccal sulcus between the mesocusps and distocusps is exaggerated by 1 or 2 small tubercles at its gingival end. The lingual sulcus resembles the lingual sulci of the maxillary first molar but is slightly larger. The mesial surface is broad, convex, and overlapped by the disto-buccal corner of the first molar. The distal surface is broad and flat and overlaps the approximating surface of the third molar. The tooth has 4 roots: 2 buccal and 2 lingual, the lengths of which are approximately 2½ times the height of the crown.

The maxillary third molar shows further modification of the basic molar pattern. Both the paracusps and distocusps are much reduced; the lingual paracusp is absent. The mesocusps are prominent, sharply pointed, and show a slight mesio-distal increase in size. They are separated by a deep oval fossa. The distocusps have lost their characteristic cusp form and are much reduced in size, but they can still be distinguished as a cuspal pair separated from one another by a deep pit-fossa. A small H-ridge connects the paired

TABLE IV

Measurements of the molar teeth of Syrian hamsters in millimeters*

	BUCCO-LINGUAL DIMENSIONS			MESIO-DISTAL DIMENSION	TOTAL TOOTH LENGTH
	Paracusps	Mesocusps	Distocusps		
Maxilla					
1st molar	1.13 ±0.04	1.30 ±0.03	1.35 ±0.02	2.33 ±0.03	3.2
2nd molar		1.39 ±0.05	1.26 ±0.05	1.77 ±0.06	3.2
3rd molar		1.35 ±0.05	1.00 ±0.04	1.56 ±0.03	2.7
Mandible					
1st molar	0.82 ±0.05	1.04 ±0.04	1.15 ±0.04	2.11 ±0.08	4.1
2nd molar		1.28 ±0.02	1.28 ±0.04	1.89 ±0.03	3.9
3rd molar		1.39 ±0.04	1.16 ±0.05	1.96 ±0.05	2.9

* Each datum represents an average measurement of 15 to 20 teeth.

mesocusps and distocusps and divides the sulcus between them. The buccal sulcus is very small, and its depth is exaggerated by a tubercle which may be so poorly defined that it appears as a small ridge. The lingual sulcus resembles the same sulci on the other maxillary molars, although it is smaller. The mesial surface is broad and convex and is overlapped by the disto-buccal corner of the second molar. The distal surface is small and evenly rounded. The tooth has 3 short, distally curved roots: buccal, lingual, and distal, which are about 1½ times the crown height.

The mandibular molars (*fig. 7*) are characterized by several distinctive features not typical of the maxillary teeth. The basic pattern is modified in the second and third molars by a reduction in size of the paracusps and a concomitant increase in size of the mesocusps, but the reduction is not so advanced as in the maxillary teeth. Both buccal and lingual paracusps remain distinct entities on each molar. The lingual cusps are higher than the buccal, and all the cusps tend to incline slightly mesialward, but the tendency is more pronounced in the lingual cusps. The buccal surfaces are of slightly greater size than the lingual.

The buccal sulci are broad, deep, longer than the lingual, and are terminated gingivally by a small ledge or minute tubercles found on the second and third molars. The lingual sulci are shorter and more narrow than the buccal and are ended by a small ridge. Broad contact areas are formed by the mesial surfaces of the second and third molars slightly overlapping the disto-buccal corners of approximating teeth. The long axes of the teeth are inclined lingually and mesially. Each tooth has 2 roots, mesial and distal.

The mandibular first molar has 6 cusps arranged in the basic molar pattern. The paracusps are very small, closely fused, and separated at their tips by a minute groove. The mesocusps resemble the paracusps but are somewhat larger. The distocusps are the largest on the crown and are separated by a well developed groove which curves into the disto-lingual corner of the crown and accentuates the distal ridge. Due to the small size and close approximation of the paracusps and mesocusps, the H-ridge system between them is not so well defined as it is between the mesocusps and distocusps. However, the connecting ridges remain very prominent. The buccal and lingual sulci are nearly equal in size and are terminated gingivally by minute ridges. The distal surface has a marked bucco-lingual convexity and a slight distal inclination. Broad contact is made with the second molar at the disto-buccal corner. The length of the roots is approximately 3 times the crown height.

The mandibular second molar has 4 major cusps and 2 reduced paracusps. The lingual paracusp is rudimentary; the buccal paracusp is small but distinct. The mesocusps are not only the largest on the second molar but are larger than any on the first. They are separated by a deep fossa. The distocusps resemble the same cusps on the first molar. Although the H-ridge between the paracusps and distocusps is poorly defined, that between the mesocusps and distocusps is easily distinguished. Since the buccal paracusp is of greater size than the lingual, the buccal sulcus between it and the mesocusp is larger than the corresponding lingual sulcus, and the entire buccal surface of the crown is somewhat greater in area than the lingual. The buccal sulcus between the mesocusps and distocusps is broad, deep, and may be slightly invaginated or divided by a small enamel ridge extending from the distocusp into its floor. Very minute tubercles may be found at the gingival end of this sulcus. The lingual sulcus resembles the corresponding sulcus of the first molar. The mesial surface is mesially inclined and slightly overlaps the disto-buccal aspect of the first molar. The distal surface resembles that of the first molar. Root lengths are approximately 2½ to 3 times the height of the crown.

The mandibular third molar is modified in the same manner as the second but to a greater degree. The paracusps are more reduced in size. However, these cusps may be separated occlusally by an extremely minute depression in the mesio-distal unit of the H-ridge. This ridge is better defined than on the second molar. Except for an increase in the bucco-lingual width of the mesocusps and a reduction in the dimensions of the distocusps, the coronal pattern is essentially as described for the second molar. The roots are about 1½ times the crown height and terminate close to the lingual side of the posterior end of the incisor.

HISTOLOGY

Histologically the molar teeth of the hamster resemble those of many higher animals (*fig. 8*). The teeth are composed primarily of dentin of typical mammalian structure. When newly erupted, the crowns are completely covered by enamel, the roots by a thin layer of cell-free cementum. As the teeth are worn by attrition, exposed primary dentin becomes metamorphosed, secondary dentin is deposited on the underlying pulpal wall, and secondary cementum is progressively deposited at the root apices.

COMMENTS AND CONCLUSIONS

The Syrian hamster, now employed as a laboratory animal in the study of dental caries, has a dentition similar but not identical to that of the albino rat. Although the incisors essentially resemble the incisors of the albino rat, the molar teeth are smaller and have a distinctly different coronal pattern (*fig. 9*). The cusps are completely covered by enamel at the time of eruption, whereas, albino rat molars are formed with enamel-free areas on cuspal tips (10). From

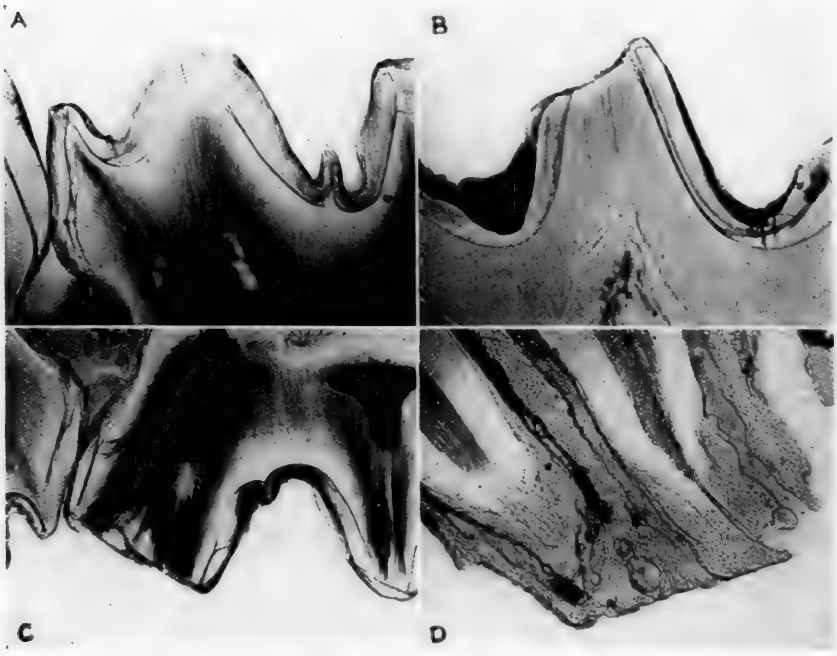

Fig. 8. Photomicrographs: A. Mandibular 2nd molar showing complete enamel covering of cusps. B. Attrition of mesial slope of mesocusp in mandibular 1st molar. Secondary dentin has been deposited on pulpal wall. Note lamella-like structures at floor of sulcus. C. Attrition of distal slope of distocusp in maxillary 1st molar showing metamorphosis of primary dentin and deposition of secondary dentin. D. Apical region of mandibular 2nd molar showing deposition of secondary (cellular) cementum.

a buccal or lingual aspect, the long axes of hamster cusps appear but slightly deviated from the long axis of the tooth. In contrast, the axes of rat molar cusps are inclined at a marked angle to the long axis of the tooth. This condition and a slight inclination of the molars result in overhanging cuspal walls.

Occlusion in the hamster is characterized by cuspal interdigitation. Functional attrition begins on the mesial slopes of the mandibular cusps and on the distal slopes of the maxillary cusps and gradually spreads over the occlusal surface. In the rat occlusion is not characterized by interdigitation. Func-

tional attrition begins on the tips of the cusps, increases the extent of enamel-free areas, and renders the overhanging cuspal walls prone to fracture (11).

In view of these differences, the molar teeth of the Syrian hamster appear to be more analogous to human teeth than those of the albino rat. Morphologically the crowns appear to favor the retention of fine food particles which might render them susceptible to a carious process comparable to that found in man.

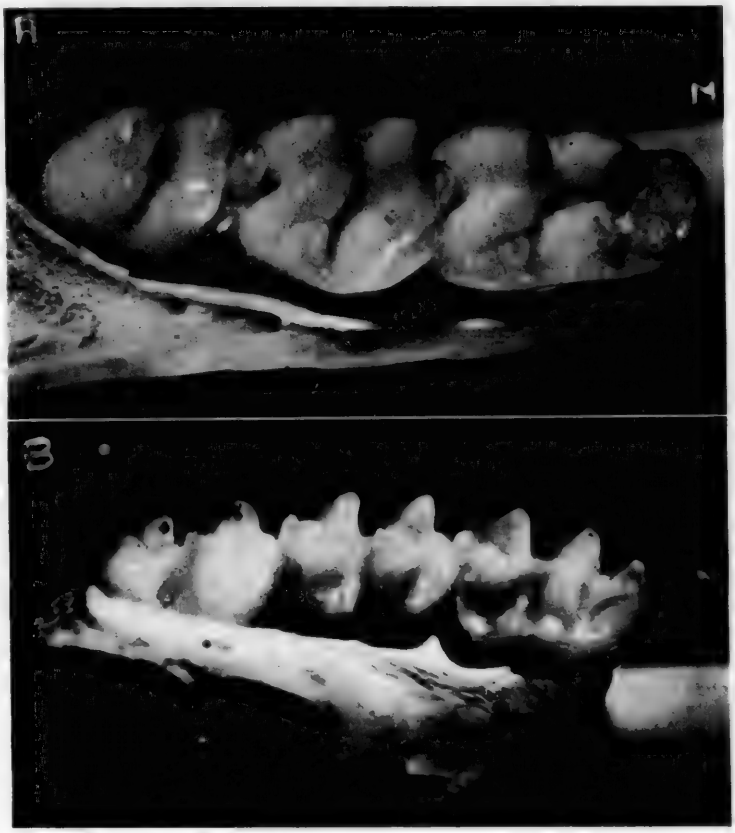

Fig. 9. Comparison of rat (A) and hamster (B) mandibular molars, magnification equal. Note difference in size, contour, and cusp inclination.

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REFERENCES TO LITERATURE

1. Arnold, F. A., The Production of Carious Lesions in the Molar Teeth of Hamsters (C. auratus), *Pub. Health Rep.*, **57**: 43, 1942.
2. Rosebury, T., The Problem of Dental Caries in Gordon's *Dental Science and Dental Art* (Philadelphia, Lea & Febiger: 1938), pp. 269–326.

3. Dale, P. P., Lazansky, J. P., and Keyes, P. H., Preliminary Observations on the Production and Inhibition of Dental Caries in Syrian Hamsters, *J. D. Res.*, **23:** 445, 1944.
4. Waterhouse, Observation on Rodentia, *Mag. Nat. Hist. III*, 274, 1839.
5. Waterhouse, Description of a New Species of Hamster, *Proc. Zool. Soc. Lond.*, **III:** 57, 1839.
6. Bruce, H. M., and Hindel, E., The Golden Hamster, Cricetus (Mesocrecitus) auratus. Notes on Its Breeding and Growth, *Proc. Zool. Soc. Lond.*, **I:** 361–366, 1934.
7. Deanesley, Ruth, The Reproduction Cycle of the Golden Hamster (C. auratus), *Proc. Zool. Soc. Lond.*, **108:** ser A, 31–37, 1938.
8. Peczenik, O., The Induction of Pregnancy in the Golden Hamster during the Breeding Pause, *Proc. Roy. Soc. Edin.*, 61 pt 3: 368–374, 1942.
9. Osborn, H. F., *Evolution of Mammalian Molar Teeth*, 103–104 (New York, Scribners: 1907).
10. Addison, W. H. F., and Appleton, J. L. T., On the Development of the Ameloblasts of the Molars of the Albino Rat with Special Reference to the Enamel Free Areas, *Anat. Rec.*, **21:** 43, 1921.
11. Bibby, B. G., and Sedwick, H. J., Formation of Cavities in the Molar Teeth of Rats, *J. D. Res.*, **13:** 429–441, 1933.

A METHOD OF RECORDING AND SCORING GROSS CARIOUS LESIONS IN THE MOLAR TEETH OF SYRIAN HAMSTERS[1]

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As the Syrian hamster appears to be well suited for investigations of experimental dental caries (1, 2), a method to record and numerically evaluate the gross caries experience in the molar teeth of this animal has been devised. The method provides a graphic record of the lesions, indicates the number of cavities and teeth affected, and offers a numerical score proportional to the amount of tooth structure destroyed. Careful compilation of these records permits comparison of individual animals and groups of animals, and facilitates interpretation and evaluation of data.

In the hamster carious lesions may involve any portion of the coronal surface of either maxillary or mandibular molar teeth. A crown may contain more than 1 cavity or be entirely destroyed. Gross lesions of 2 types may be readily distinguished: 1. those occurring in sulci, occlusal grooves, and fossae, and 2. those occurring in smooth surfaces either interproximally or cervically. A successful charting and scoring technic must provide for the possible occurrence of large and small lesions of both types.

The recording system described is based on the following premises: 1. that gross lesions seen in molars at 14 to 20 diameters represent true dental caries (2), 2. that the etiology of all lesions is essentially the same throughout the molar dentition, and 3. that the quantity of tooth substance destroyed is proportional to the activity of the carious process and may be used as the basis for numerical score estimation.

METHOD

The molar teeth of the hamster are characterized by pointed cusps separated from one another by bucco-lingual sulci and occlusal fossae and grooves (3). Major cusps are well defined, and for purposes of scoring, may be considered individual entities. One cusp may be selected as a standard in size and given an arbitrary numerical value, e.g. the disto-buccal cusp of the maxillary first molar may be assigned a value of 6. With reference to this cusp, all cusps in the dentition may be proportionally evaluated according to their relative sizes. The total value of a crown becomes the sum of the values of its cusps. Using this value, scores for carious lesions can be estimated from the fraction of crown substance destroyed and expressed numerically as the product of the fraction and the total crown value.

Destruction of a single cusp or an obvious fraction ($\frac{1}{4}$, $\frac{1}{2}$, $\frac{3}{4}$) of a crown entails a simple

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scoring procedure. However, the presence of several lesions of different size on one crown requires a more detailed method. To facilitate and simplify the process of evaluation in the latter case, the buccal, lingual, and occlusal surfaces of the molars are divided into a

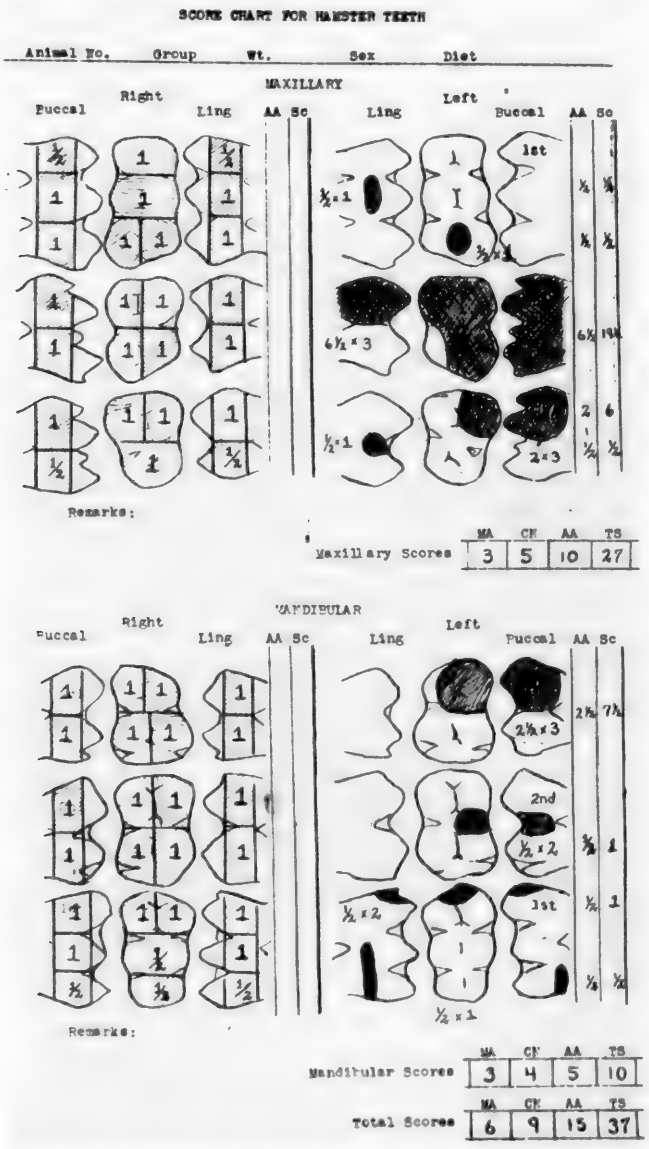

Fig. 1. Score chart for hamster teeth. Right dentition indicates unit area divisions, left shows cavity outlines and scores.

number of "units" of area which are, for the most part, of about equal size. These areas are given a value of 1 or ½ according to their approximate extent (*fig. 1*, right dentition and, Table I).

To score a cavity, it is necessary to make 2 estimations: 1. the number of "unit areas" affected, and 2. the depth of the cavity. As precise measurements of cavity dimensions are impractical, cavity depth is estimated in whole numbers 1, 2, or 3. These figures represent approximate dimensional values: 1 is equivalent to a depth of 0.1 to 0.2 mm.; 2, a depth of 0.2 to 0.4 mm.; and 3, a depth of 0.4 to 0.6 mm. The score is the product of the number of unit areas affected and the figure representing depth. Theoretically cavity scores are volumetric estimations expressed in arbitrary values proportional to the amount of tooth substance destroyed. If correctly derived, they should be equal to that obtained by fractional estimation.

By deduction, the total score value for any crown is the product of the sum of its unit areas × 3 as indicated in Table I. The proximal surfaces are not divided into unit areas because to include them would reintroduce dimensions considered in the combined value of area and depth.

CHARTING AND SCORING

A mimeographed form is used to chart and score all lesions (*fig. 1*). The animal number, its group, weight, sex, and other pertinent data are noted at the top of the record. The jaws are fixed in 10% formalin, washed, dried and stripped of soft tissue. Each tooth is carefully examined at 14 to 20 diameters magnification. A fine explorer is used to remove debris and to determine the full extent of each cavity. Lesions are then outlined on the chart. Typical cavities are indicated in *fig. 1*, left dentition. Mesial and distal tooth surfaces are not charted because they are rarely affected without the involvement of buccal, lingual, or occlusal surfaces. When a cavity involves several surfaces including the mesial and distal, it is outlined as illustrated for the maxillary left second and third and the mandibular left third molar. Lesions too small to be positively identified may be charted, but are not included in cavity counts or scores. However, these questionable lesions may be noted under remarks and sectioned for histological study if desired.

After the lesion is charted, the sum[2] of the affected unit areas and fractions thereof are recorded beside the cavity outline and in column AA (areas affected). For practical purposes, fractional involvements of a unit are rarely rated less than ¼. A depth value is then assigned and the appropriate figure is placed next to the value for unit areas beside the outlined cavity (*fig. 1*). The score is computed as described and recorded in the column "Sc" (score). When an approximal surface is involved alone, it is necessary to break down the overlying tooth structure to determine the extent of the lesion. This procedure establishes an occlusal cavity which can be rated in the usual manner.

Individual scores are added to obtain total scores. Four values are recorded for each jaw and the entire dentition: 1. the number of molars affected (MA); 2. the number of cavities (CN); 3. the number of unit areas affected (AA); and 4. the total score (TS). On the basis that a score of 282 indicates com-

[2] An outline of the unit areas simplifies summation until one is familiar with the unit divisions.

plete destruction of the molar dentition (Table I), a caries index can be determined as the percentage of tooth substance destroyed, e.g. score 37 equals 37/282 equals 13.1%.

TABLE I

Unit areas and whole crown values employed in scoring

	UNIT AREAS*	×	GREATEST DEPTH VALUE	=	WHOLE CROWN VALUES†
Max 1st Molar	9		3		27
Max 2nd Molar	8		3		24
Max 3rd Molar	6		3		18
Mand 1st Molar	8		3		24
Mand 2nd Molar	8		3		24
Mand 3rd Molar	8		3		24
	47				141

* 47 unit areas × 2 equals 94 unit areas per molar dentition.

† 141 × 2 equals 282 equals total molar score (Caries score representing complete destruction).

DISCUSSION

The recording method described incorporates several advantages in obtaining data on hamster caries. It is easy to use and requires no extraneous or laborious technics. The location, type, and extent of lesions are graphically illustrated. This facilitates scoring, permits rechecking of observations by different investigators, and provides permanent records. The scoring system represents an attempt to evaluate caries experience on a consistent and standardized basis which depends directly on the quantity of tooth substance destroyed regardless of location.

Estimations of areas affected is a partial indication of the extent of tooth substance destroyed and resembles the system of Cox, Dodd, Dixon and Matuschak (4) in that the number of areas affected is related to cavity size and caries incidence. Although proximal surfaces are not considered, this is a consistent omission that appears to be no greater than those for other systems using selective areas (4, 5) or limited numbers of teeth (6). As the extent of cavitation is not fully indicated by the number of unit areas affected, provision is made to evaluate depth.

Cavity scores are theoretically linear, and may be added to approximately estimate the extent of destruction. The greater the amount of toothstructure destroyed, the easier and more accurately are estimations made. Although a small amount of tooth substance must be sacrificed to score cavities affecting interproximal surfaces alone, these cavities are so rare and the amount of tooth substance sacrificed so small, that the effect on the final results is not appreciable. The range of error in scores of small cavities may be considerable without significantly influencing the total score.

As molar crowns do not readily lend themselves to equal division, any system

based on this principle is arbitrary and limited. Examination of the molars reveals that all the unit areas rated as "1" are not of equal size. This discrepancy is particularly apparent in mandibular molars. However, its effect is somewhat compensated by the fact that scoring values are comparatively high in one instance and low in another.

Since experimentally produced caries in the teeth of laboratory animals is an important phase of dental research, a satisfactory method of evaluating caries experience is a necessity. The extent to which comparisons and conclusions can be made depends upon the nature of the carious process and the method of evaluation.

Scoring systems for experimental caries have been devised for use with the rat (4, 6). However, caries activity in the albino and hooded rat seems to be more related to the physical nature of cereal constituents of the diet than to nutritional ingredients (6, 7, 8) and shows a definite predilection for the mandibular teeth (7, 8, 9, 10). These facts increase the problem of scoring and evaluating the decay. Although caries activity in the rat frequently has been estimated without benefit of histological examination, results thus determined represent both "fracture" and "fissure" caries. When teeth are extensively involved, determination of etiology and point of origin are extremely difficult if not impossible. Since only lesions in which fracture has played no part may be comparable to those observed in human teeth, a completely satisfactory evaluation of the caries experience requires histological study (4, 6).

Use of the hamster seems to eliminate many of these complications. Caries activity in this animal does not depend upon coarse cereals in the diet and is well distributed in maxillary and mandibular molar teeth (1, 2). Since fracture apparently is not a factor in production, all macroscopic (× 14) lesions may be assumed to represent the type of dental caries found in man. Questionable defects that may represent incipient caries are not considered in the scores because the consistent omission of such lesions does not entail significant loss in interpretation of the results. If caries susceptibility is uniformly distributed in the experimental groups, the extent of cavitation would be proportional to the activity of the carious process, and scores derived from the evaluation of gross lesions assumed valid and comparable.

SUMMARY

A method of recording and scoring carious lesions in the molar teeth of the Syrian hamster has been described. The technic provides a permanent record of lesions and caries scores representative of the amount of tooth structure destroyed.

REFERENCES TO LITERATURE

1. Arnold, F. A., Production of Carious Lesions in the Molar Teeth of Hamsters (C. Auratus), *Pub. Health Rep.*, **57**: 43, 1942.
2. Dale, P. P., Lazansky, J. P., and Keyes, P. H., Preliminary Observations on the Production and Inhibition of Dental Caries in Syrian Hamsters, *J. D. Res.*, **23**: 445, 1944.

3. KEYES, P. H., AND DALE, P. P., A Preliminary Survey of the Pouches and Dentition of the Syrian Hamster, *J. D. Res.*, **23**: 427, 1944.
4. COX, G. J., DODDS, M. L., DIXON, S. F., AND MATUSCHAK, M. C., Experimental Dental Caries. III A System of Recording Corn Meal Caries in Rats, *J. D. Res.*, **18**: 469, 1939.
5. BODECKER, C. F., AND BODECKER, H. W. C., A Practical Index of the Varying Susceptibility to Dental Caries in Man, *D. Cosmos*, **73**: 707, 1931.
6. ROSEBURY, T., KARSHAN, M., AND FOLEY, G., Studies in the Rat of Susceptibility to Dental Caries, *J. A. D. A.*, **21**: 1599, 1934.
7. HOPPERT, C. A., WEBBER, P. A., AND CANNIFF, T. L., Production of Dental Caries in Rats Fed an Adequate Diet, *J. D. Res.* **12**: 161, 1932.
8. ROSEBURY, T., KARSHAN, M., AND FOLEY, G., Studies in the Rat of Susceptibility to Dental Caries, *J. D. Res.*, **13**: 379, 1933.
9. BIBBY, B. G., AND SEDWICK, H. J., Formation of Cavities in Molar Teeth of Rats, *J. D. Res.*, **13**: 429, 1933.
10. MILLER, B. F., Inhibition of Experimental Dental Caries in the Rat by Fluoride and Iodoacetic Acid, *Proc. Soc. Exper. Biol. Med.*, **39**: 389, 1938.

PRODUCTION AND INHIBITION OF DENTAL CARIES IN SYRIAN HAMSTERS[1, 2]

Preliminary Observations

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Recently, Arnold (1) reported that the molar teeth of the Syrian hamster, *Cricetus auratus*, became carious when the animal was fed an adequate diet of finely ground corn or a coarse corn ration similar to that of Hoppert, Webber, and Canniff (2). Two distinct types of lesions were described, those occurring in occlusal fissures and those that developed in cervical areas of proximal surfaces, particularly in maxillary and mandibular first molar teeth.

Investigations of the etiology of dental caries have been handicapped by lack of a laboratory animal that would develop lesions comparable to those found in man. Because of the implications apparent in Arnold's findings, it seemed important that certain of his results be confirmed by independent investigation, and that the influence of fluorine, found effective in limiting caries in man and lower animals (3), be tested in hamsters.

EXPERIMENTAL

Sixty-four hamsters raised from pure inbred stock obtained from the National Institute of Health were the subjects of study. From weaning, 25th day, to the beginning of the experiment, 40th day, the animals were maintained on commercially prepared (Purina) rabbit chow pellets. Litter mates, male and female, were distributed into 4 groups of 16 animals and fed various unrestricted diets throughout an experimental period of 125 days. The average weight of the animals at the beginning of the experiment was 60 grams.

Rations assigned to individual groups follow:

I. Coarse corn meal (20 mesh), 60 percent; whole powdered milk, 30 percent; alfalfa meal, 3 percent; sodium chloride, 1 percent; and linseed meal (when available, chiefly after the first 50 days), 6 percent. Fluorine: approximately 0.4 p.p.m. (parts per million).

II. Same as I except that 60 mesh instead of 20 mesh corn was used.

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[2] This work was made possible by a grant of the Carnegie Corporation of New York and the Eastman Dental Dispensary of Rochester, New York.

[3] Now on duty, A. U. S., Dental Corps.

[4] Now at Tufts Dental College, Boston, Mass.

III. Commercially prepared (Purina) rabbit chow pellets containing a minimum of 14.5 percent of protein, 44.0 percent of carbohydrate, and 2.0 percent of fat; and a maximum of 18.0 percent of crude fiber. Fluorine: 56.5 to 78.8 p.p.m., average 68.3 p.p.m.[5]

IV. Ration I supplemented by 50 p.p.m. of fluorine as NaF in the drinking water.

Figures for fluorine content represent average results of duplicate analyses of 50-gram samples of rations I and II, and of eight 15-gram samples of ration III by a modification of the method of Willard and Winter (4). All animals were weighed weekly and provided with suitable supplements of fresh apples, carrots, and lettuce.

On completion of the experimental period the animals were sacrificed, the jaws removed, fixed in 10% formalin, washed, dried, and stripped of soft tissues. The molar teeth of both jaws were carefully examined with a fine pointed explorer under a binocular (× 14) microscope. Carious lesions were graphically recorded

TABLE I

Caries activity in Syrian hamsters

Group	I	II	III	IV
Ration	Coarse corn, water	Fine corn, water	Rabbit chow pellets, water	Coarse corn, 50 p.p.m. F as NaF in water
Animals	16	10	15	10
Caries-free animals	0	0	4	4
Molar teeth examined	192	120	180	120
Carious molars	80	77	26	11
Maxillary	32[S]	44[S]	11	4
Mandibular	48	33	15	7
Units of area* affected	192	155	40	24
Average total caries score per animal	31.2	36.5	5.2	5.8

* The molar dentition of the hamster contains 94 units of area.

[S] Difference between the number of carious molars in maxilla and mandible is statistically significant.

on a specially designed chart and scored independently by 2 investigators with closely agreeing results. The identity of questionable defects was established in histological ground sections. The method of scoring provided for each lesion a numerical caries score indicative of destruction in 3 dimensions (units of tooth surface destroyed × a number representative of cavity depth). The sum of the scores of all the lesions in an animal yielded a *total caries score* for that animal. The total scores of the animals in each group were averaged and the resultant data presented with other findings in Table I.

FINDINGS

Dental decay was observed in hamsters of all groups (Table I). No animal that received a fluoride-free ration was entirely exempt of caries. Hamsters

[5] A communication from the author (10/3/1944) remarks that this was an exceptionally high amount of fluorine and that subsequent analyses of new shipments of the chow contained 3 to 8 p.p.m. of fluorine. This shows the advisability of repeated analyses.

fed coarse (I) and fine (II) corn diets had the highest average total caries scores (31.2 and 36.5 respectively). Average total scores of groups maintained on rabbit chow pellets (III) and the fluoride-supplemented diet (IV) were reduced to 5.2 and 5.8 respectively. Four animals in each of the latter groups were free from carious lesions.

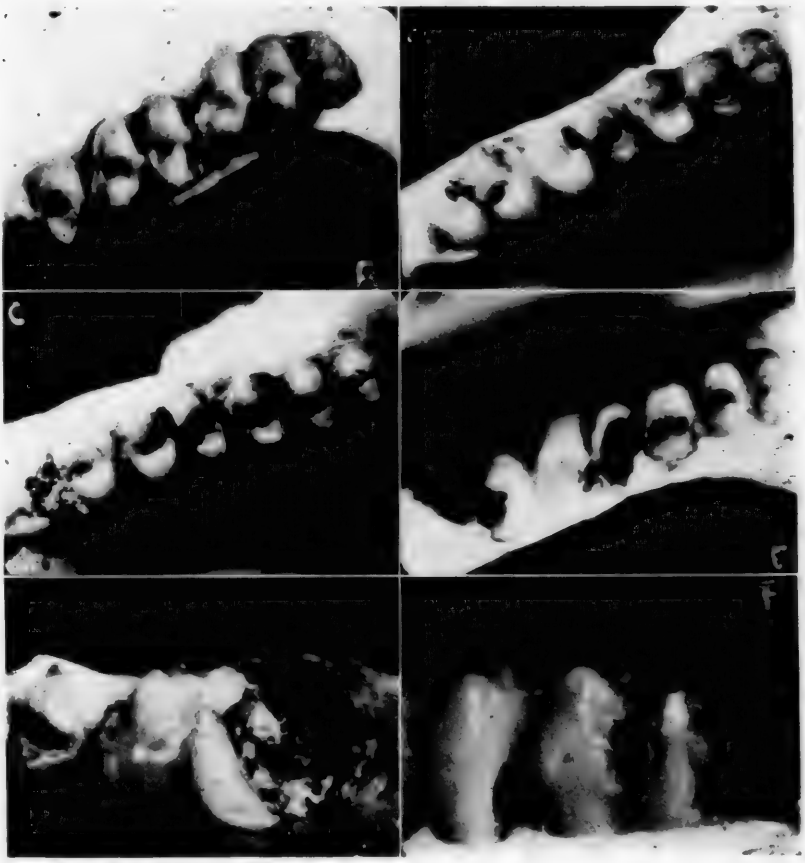

Fig. 1. Maxillary Teeth: A. Normal, occlusal view. B. Incipient occlusal caries. C. and D. Advanced occlusal caries. E. Destruction of 3rd molar crown. F. Enamel undermined by lesion in lingual sulcus of 3rd molar tooth.

In contrast to the mandibular preference of dental caries observed in rats, decay was found to a large extent in the maxillary molar teeth. Animals fed the fine corn diet (II) revealed a high degree of caries activity in maxillary as compared with mandibular teeth, but a reverse tendency was noted in animals that received coarse corn (I) or rabbit chow (III). Fluorosis, manifested by loss of pigment in incisor enamel, became grossly evident after 10 weeks on diets

III and IV. However, pigmentary changes resulting from fluoride ingestion as well as normal pigmentation showed considerable variation.

Gross (*figs. 1 and 2*) and microscopic (*fig. 3*) examinations of the lesions revealed characteristics strikingly similar to those of caries in man, including typical enamel undermining and production of yellow pigment. Lesions were most prevalent in deep occlusal grooves and in buccal and lingual sulci. To a far lesser extent, they occurred at the gingival margins of the crown. Interproximal caries was occasionally observed where food particles and debris were found wedged between the teeth. Some teeth were attacked simultaneously at several points. Completely destroyed crowns were a common finding.

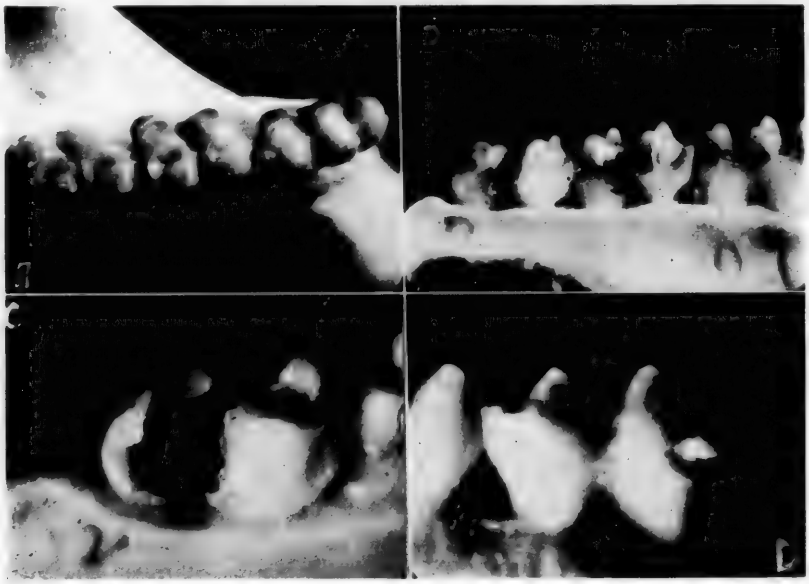

Fig. 2. Mandibular Teeth: A. Normal. B. Caries originating in buccal sulci. C. Extensive occluso-buccal involvement of 3rd molar tooth. D. Cervical caries of mesiobuccal angle in 1st molar tooth.

DISCUSSION

In general, the results confirm those of Arnold (1). Caries in hamster molar teeth developed in areas where food is likely to accumulate, i.e. in deep buccolingual sulci and occlusal fossae, and at cervical margins. As many as 3 or 4 lesions appeared simultaneously in some teeth, presumably because of the large number of vulnerable areas provided by greatly accentuated grooves and sulci. Enamel fracture was apparently of minor importance in the production of cavities. Lesions were observed in teeth without fracture, and were common in animals that received a finely ground corn diet.

Observation of substantial numbers of carious lesions in the maxillary teeth of the hamster appears to differentiate caries activity in this animal from that

in rats. Mandibular preference of caries in the latter has been reported by Hoppert, Webber, and Canniff (2), Bodecker and Applebaum (5), Bibby and Sedwick (6), Rosebury (7), Templin and Steenbock (8), Miller (9), McClure and Arnold (10), and Armstrong (11). However, Hodge and Finn (12) in a

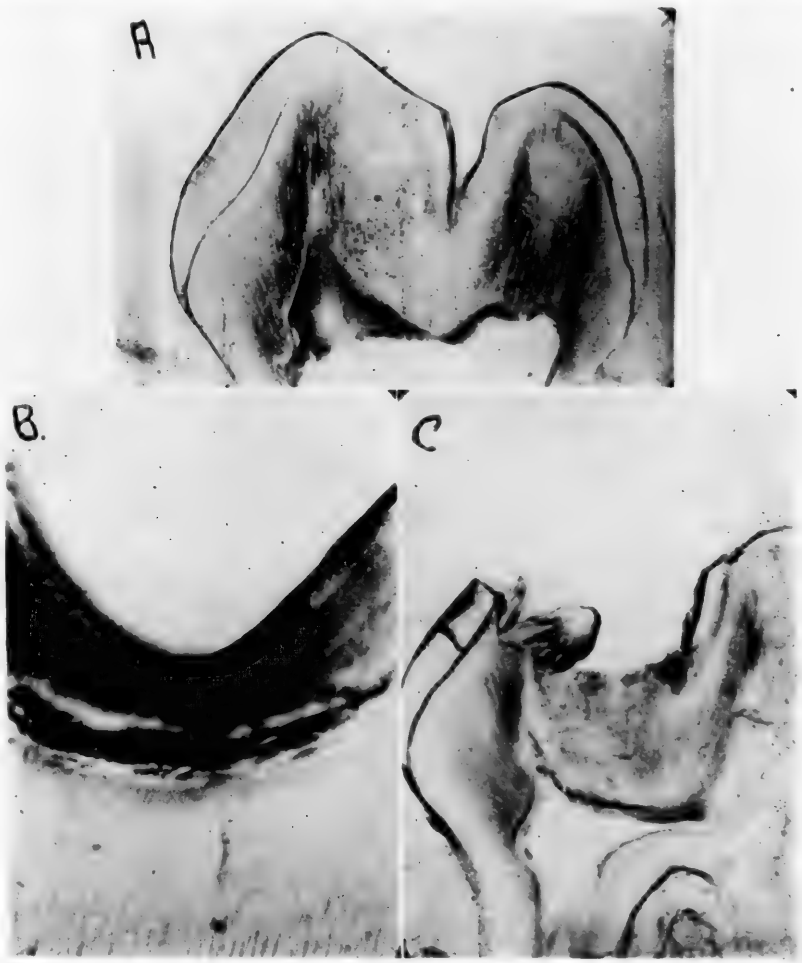

Fig. 3. Ground section photomicrographs of normal and carious hamster molar teeth: A. Intact. B. Incipient lesion at floor of sulcus; enamel undermined at base of lamella. C. Occlusal lesion with some evidence of secondary dentin formation.

carefully controlled experiment found a high incidence of experimentally produced caries in the maxillary teeth. Doubt exists regarding the true situation and the factors involved, but on the whole, the difference between hamsters and rats in respect to the distribution of carious lesions seems amply supported.

The difference between the number of maxillary and mandibular carious molar teeth is highly significant statistically in group I, less so in group II, and insignificant in group III and IV animals.

The decrease in caries susceptibility of group III animals was apparently a product of the fibrous consistency and high fluorine content (average 68.3 p.p.m.) of their ration. The amount of fluorine in the coarse (I) and fine (II) corn diets was considerably lower (0.4 p.p.m.) and of the same order as obtained for common cereals by Machle, Scott, and Treon (13).

Thirteen animals died before completion of the experiment, 2 from intestinal obstruction due to ingestion of sugar cane bedding, 11 from indeterminate causes. Fatalities included 6 hamsters receiving supplementary fluorine in the drinking water, but the halogen apparently was not a responsible factor inasmuch as animals on supplement-free diets fared as badly. It is our experience that 50 p.p.m. of fluorine as NaF in the drinking water is well tolerated by Syrian hamsters. Data from diseased animals were discarded to avoid ambiguity in interpretation of the results.

Examination of the incisor teeth did not indicate whether the hamster required greater amounts of fluorine than the rat to produce pigmentary changes in the enamel since the former consumes approximately a third as much water as the rat of comparable weight.

SUMMARY

Preliminary studies of dental caries in Syrian hamsters indicate that it is grossly and microscopically comparable to the disease in human beings. Unlike caries produced in the rat,[6] lesions were widely distributed in both maxillary and mandibular molar teeth and developed without evidence of fracture. As much as 50 p.p.m. of fluorine as NaF in the drinking water and upward of 68 p.p.m. of the halogen in the food were tolerated without deleterious effects and exerted a caries-inhibiting effect. The type of caries produced in the hamster and the adaptability of the animal to the laboratory seem to render it superior to the rat[4] as a subject for caries investigation.

The authors are indebted to assistants for fluoride analyses of the rations and statistical evaluation of the data, and to Mr. Ray Kesel for care and breeding of the animals.

REFERENCES TO LITERATURE

1. Arnold, F. A., Production of Carious Lesions in the Molar Teeth of Hamsters (C. auratus), *Pub. Health Rep.*, **57:** 43, 1942.
2. Hoppert, C. A., Webber, P. A., and Canniff, T. L., Production of Dental Caries in Rats, *Science*, **74:** 77, 1931. Also: *J. D. Res.*, **12:** 161, 1932.
3. American Association for the Advancement of Science, Publication No. 19, Fluorine and Dental Health (Lancaster: Science Press, 1942).
4. Willard, H. H., and Winter, O. B., Volumetric Method for Determination of Fluorine, *Ind. and Eng. Chem., Anal. Ed.*, **5:** 7, 1933.

[6] The albino and hooded rats have commonly been used in caries' studies. See "Dental Caries in the Cotton Rat" by Shaw, Schweigert, Elvehjem and Phillips (*J. D. Res.*, **23:** 417, 1944, this issue). Ed.

5. Bodecker, C. F., and Applebaum, E., Experimental Dental Caries in Rats, *D. Cosmos*, **74**: 335, 1932.
6. Bibby, B. G., and Sedwick, H. J., Formation of Cavities in Molar Teeth of Rats, *J. D. Res.*, **13**: 429, 1933.
7. Rosebury, T., Karshan, M., and Foley, G., Studies in the Rat of Susceptibility to Dental Caries, *J. D. Res.*, **13**: 379, 1933.
8. Templin, V. M., and Steenbock, H., Vitamin D and the Conservation of Calcium in the Adult. III Effect of Vitamin D on the Teeth of Rats, *J. Biol. Chem.*, **100**: 217, 1933.
9. Miller, B. F., Inhibition of Experimental Dental Caries in the Rat by Fluoride and Iodoacetic Acid, *Proc. Soc. Exper. Biol. Med.*, **39**: 389, 1938.
10. McClure, F. J., and Arnold, F. A., Observations on Induced Dental Caries in Rats, *J. D. Res.*, **20**: 97, 1941.
11. Armstrong, W. D., Review of Dental Fluorosis Studies at the University of Minnesota. American Association for the Advancement of Science, Publication No. 19. (Lancaster: Science Press, 1942), p. 62.
12. Hodge, H. C., and Finn, S. B., Reduction in Experimental Rat Caries by Fluorine, *Proc. Soc. Exper. Biol. Med.*, **42**: 318, 1939.
13. Machle, W., Scott, E. W., and Treon, J., Normal Urinary Fluorine Excretion and the Fluorine Content of Food and Water, *Am. J. Hygiene*, **29**: 139, 1939.

STATISTICAL STUDY OF THE PRIMARY CAUSES OF EXTRACTIONS[1]

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This study was undertaken in an attempt to evaluate the relative importance of the common dental disorders. Caries and pyorrhea are considered the most common dental diseases, but the literature is somewhat confusing as to their comparative importance. Caries is said to be the most prevalent of all diseases (1), even more common than the common cold (2). Very few individuals escape its attack, its occurrence ranging from 75% to 95% in the civilized nations (3). Yet the fact has been emphasized that periodontal disease is extremely prevalent (4), with an estimated prevalence of 80% among adults (2). And it has been suspected as the cause of more extractions than any other disease (5). It is hoped that this study will present a clearer picture of the problem.

METHOD

The investigation consisted of the study of 1,167 consecutive registrants at the University of Michigan dental school clinic. About one-third of these underwent extractions. Using the diagnoses as made by staff members, the primary causes of extractions were divided into 8 groups:

1. *Caries.* All extractions in which caries was the primary factor, inoperable pulpal involvements due to caries, teeth fractured due to extensive caries, pulpless teeth (as a result of caries) with apical pathosis, etc. All but 5 of the pulpal involvements were due to caries.
2. *Periodontal disease (pyorrhea).* All teeth lost as a result of periodontal disease.
3. *Impaction.* Extractions indicated by complete or partial impaction (non-eruption).
4. *Prosthetic correction.* Teeth sacrificed solely to facilitate better prosthetic restoration.
5. *Orthodontic correction.* Teeth (except supernumerary) which were extracted for correction or prevention of malocclusion.
6. *Supernumerary teeth.*
7. *Traumatic causes.* Pulpal pathosis due to trauma, and teeth fractured (without caries) beyond safe restoration.
8. *Surgical involvement.* Teeth involved secondarily in maxillo-facial surgery.

These 8 causal groups provide the 1,424 teeth in this study. The grouping is patterned after Brekhus' report on the causes of extractions (6). However, it is felt that primary factors are more significant for present purposes than secondary causes used by Brekhus.

[1] Received for publication September 12, 1944.

[2] Graduate student.

Table I presents the distribution of extractions as to cause, sex, and age. *Fig. 1* indicates the percentage of extractions in each causal group. From these two charts, it is evident that caries and periodontal disease, together, accounted for almost 90% of the teeth lost, the remaining 10% being distributed among the 6 minor causes (Table II).

TABLE I

Distribution of extractions

AGE	SEX	NO. EXT.	NO. CASES	CARIES		PYORRHEA		IMPACTION		PROSTHET.		ORTHODONT		SUPERNUM		TRAUMATIC		SURGICAL	
				Ext.	Cases	Ext.	Cases	Ext.	Cases	Ext.	Cases	Ext.	Cases	Ext.	Cases	Ext.	Cases	Ext.	Cases
1–10	M	21	7	19	5							1	1	1	1				
	F	42	15	35	10							5	3	2	2				
11–20	M	85	63	59	44	1	1	12	8			10	7			3	3		
	F	29	20	13	9	0	0	10	6			6	5			0	0		
21–30	M	154	68	127	45	1	1	21	17	0	0	3	3	2	2			0	0
	F	135	49	81	29	20	3	15	11	13	3	1	1	2	1			3	1
31–40	M	163	30	52	18	102	7	5	3	0	0			1	1	3	1		
	F	67	18	49	14	16	2	1	1	1	1			0	0	0	0		
41–50	M	107	16	63	11	44	5			0	0								
	F	153	19	40	6	104	12			9	1								
51–60	M	152	12	2	2	135	9			15	1								
	F	119	15	68	8	51	7			0	0								
61–70	M	89	9	34	3	51	4			2	1							2	1
	F	18	3	9	2	9	1			0	0							0	0
over 70	M	61	5	37	3	24	2												
	F	29	4	7	2	22	2												
Total....	M	832	210	393	131	358	29	38	28	17	2	14	11	4	4	6	4	2	1
	F	592	143	302	80	222	27	26	18	23	5	12	9	4	3	0	0	3	1
		1424	353	695	211	580	56	64	46	40	7	26	20	8	7	6	4	5	2

Fig. 2 represents the total extractions classified according to age and sex. *Fig. 3* and Table III presents total extractions, caries-extractions, and pyorrhea-extractions, in each age group.

The average extractions per patient in each causal group are given in *Fig. 4* and Table IV:

The averages for caries and periodontal disease seem significant. Data for the other groups are included merely for the sake of completeness. Their significance is questionable because of the small number of cases.

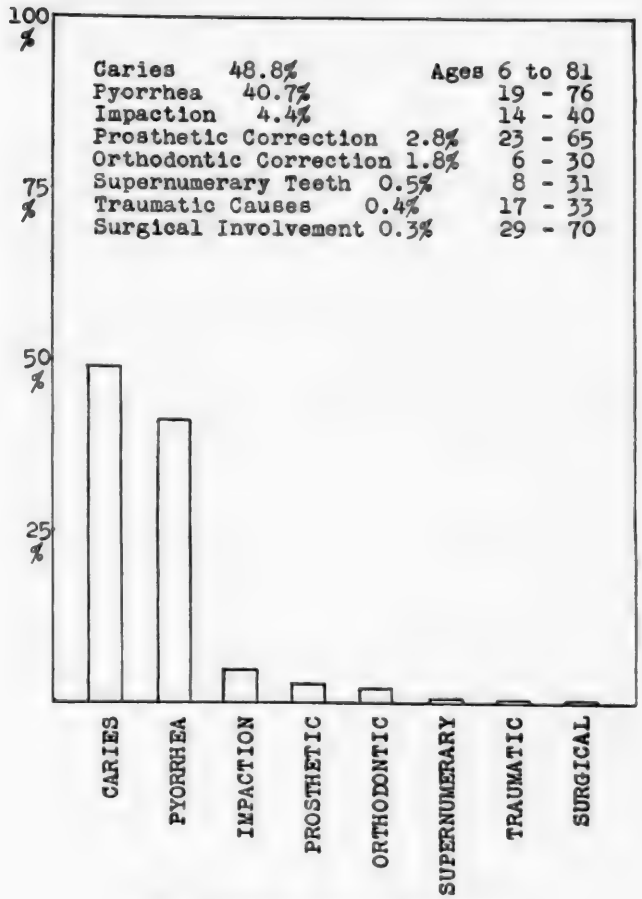

Fig. 1. Primary Causes of Extractions

TABLE II

	PER CENT	NUM. EXT.	AGE RANGE
Caries	48.8	695	6 to 81
Periodontal disease	40.7	580	19–76
Impaction	4.4	64	14–40
Prosthetic correction	2.8	40	23–65
Orthodontic correction	1.8	26	6–30
Supernumerary teeth	0.5	8	8–31
Traumatic causes	0.4	6	17–33
Surgical involvement	0.3	5	29–70

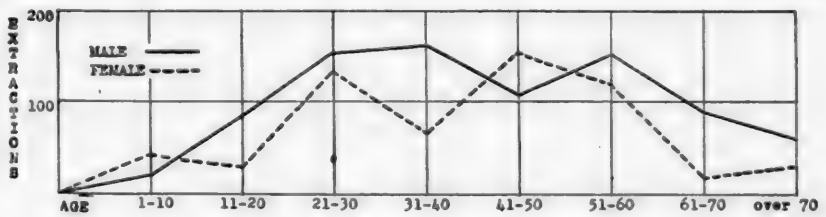

Fig. 2. Age-Sex Factor in Total Extractions

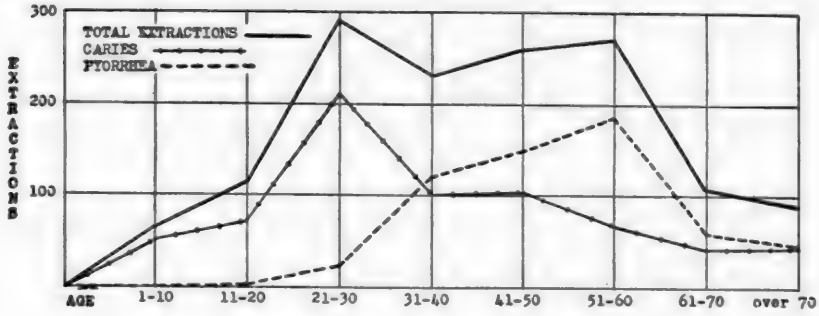

Fig. 3. Age Factor in Caries- and Pyorrhea-Extractions

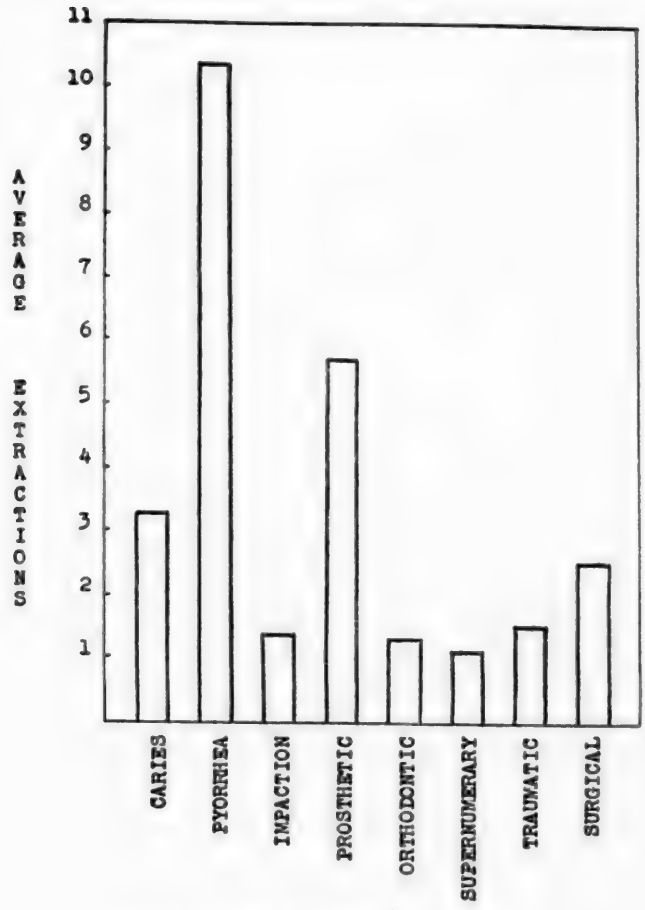

Fig. 4. Average Extractions per Patient

SUMMARY

An analysis was made of the causes of extraction of 1,424 teeth. In 59% of the patients studied, teeth were extracted as a result of caries. This comprised 48.8% of all extracted teeth. Each patient averaged 3.29 extractions. The caries group ranged in age from 6 to 81 years, the only causal group that appeared in all ages. Males outnumbered female patients, 3 to 2.

Those in the periodontal disease group comprised 13% of all patients, and 40.7% of all extractions. Each patient averaged 10.35 extractions. Numbers of male and female patients were nearly equal.

Impacted (unerupted) teeth constituted the third largest group, 4.4% of all teeth extracted, involving 3 age groups, from 14 to 40 years. Extractions for

TABLE III

AGE GROUP	CARIES EXT.	PYORRHEA EXT.	TOTAL EXT.
1–10	54	—	63
11–20	72	1	114
21–30	208	21	289
31–40	101	118	230
41–50	103	148	260
51–60	70	186	271
61–70	43	60	107
over 70	44	46	90

TABLE IV

Caries	3.29
Periodontal disease	10.35
Impaction	1.39
Prosthetic correction	5.71
Orthodontic correction	1.30
Supernumerary teeth	1.14
Traumatic causes	1.50
Surgical involvement	2.50

prosthetic correction made up the fourth group, with 2.8% of all extractions, but with less than 1% of the total patients. Supernumerary teeth accounted for 0.5% of all extractions, traumatic causes for 0.4%, and surgical involvement, 0.3%.

From this data, the most notable fact seems to be the relative importance of caries and periodontal disease. Almost one-half the extractions were due to caries, and about 40% were due to pyorrhea. Yet caries-extractions averaged approximately 3 per patient, and periodontal disease-extractions were approximately 10 per patient. Although extraction statistics such as these are entirely inadequate in determining the incidence of dental disease and disorders, they do indicate the relative importance of the various disorders in terms of oral health.

REFERENCES TO LITERATURE

1. American Dental Association Committee on Research in Dental Caries, *Dental Caries*, 2nd ed. (New York, Lancaster Press: 1941), p. 9.
2. Brekhus, P. J., *Your Teeth; Their Past, Present, and Probable Future* (Minneapolis, Univ. of Minn. Press: 1941), pp. 4, 77.
3. Bunting, R. W., and Hill, T. J., *Oral Pathology*, 2nd ed. rev. (Philadelphia, Lea and Febiger: 1940), p. 159.
4. MacNevin, M. G., and Vaughan, H. S., *Mouth Infections and Their Relation to Systemic Disease* (New York, Purcell Memorial: 1930), p. 372.
5. Merritt, A. H., *Periodontal Diseases*, 2nd ed. (New York, MacMillan: 1939), p. 1.
6. Brekhus, P. J., Dental disease and its relation to the loss of human teeth, *J. A. D. A.*, **16**: 2237, 1929.

LEUKOCYTES IN THE EPITHELIUM[1]

B. ORBAN

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In inflammatory reactions of the gingiva, leukocytes of the polymorphonuclear type migrate from the capillaries into the connective tissue and from there, through the epithelium, to the surface. Bardeleben[2] in his study of the healing process of wounds stated that a degenerative condition of the epithelium might be responsible for the extensive invasion of leukocytes into the epithelium. It is quite probable that ultimately the leukocytes destroy the epithelium. Hatton[3] also observed the extensive infiltration of the gingival epithelium by leukocytes, and considered retrograde changes in the epithelium responsible for this phenomenon.

Specimens came under observation where the epithelium was densely invaded by leukocytes (*fig. 1*) in spite of the fact that the surrounding connective tissue was in a state of chronic inflammation and more or less free of leukocytes. The connective tissue in this case is densely filled with plasma cells. In the zone immediately limiting the epithelium there are some leukocytes. The epithelium itself shows an extensive invasion of leukocytes which becomes denser close to the surface.

In higher magnification (*fig. 2*) it becomes evident that between the plasma cells there are only a few leukocytes present and these are mostly elongated, indicating active migration. In the epithelium the leukocytes are found in the intercellular spaces. Some are elongated while others have their usual round shape.

That there might be some special significance in the gathering of leukocytes in the epithelium was conceived by the study of microscopic sections of a specimen clinically diagnosed as pulp polyp. The sections show that it was not a pulp polyp but an overgrowth of granulation tissue from the highly infiltrated inter-radicular space (*fig. 3*). This granulation tissue is densely infiltrated with plasma cells; in some areas there is nothing else but plasma cells. The apex of the tooth and the surface of the granulation tissue are connected by strands of epithelial tissue (*fig. 4*). The outline of the epithelial tissue is not sharp, and only by the somewhat darker staining reaction can it be recognized as such. In higher magnification (*fig. 5*) the epithelium is seen infiltrated through and through with leukocytes, while the surrounding connective tissue is crowded

[1] Read at the meeting of the Chicago Section of the International Association for Dental Research, April 18, 1943. Received for publication September 18, 1944.

[2] Bardeleben, H. V., *Virchow's Arch.*, **163:** 497, 1901.

[3] Hatton, E. H., *D. Summary*, **39:** 85, 1919.

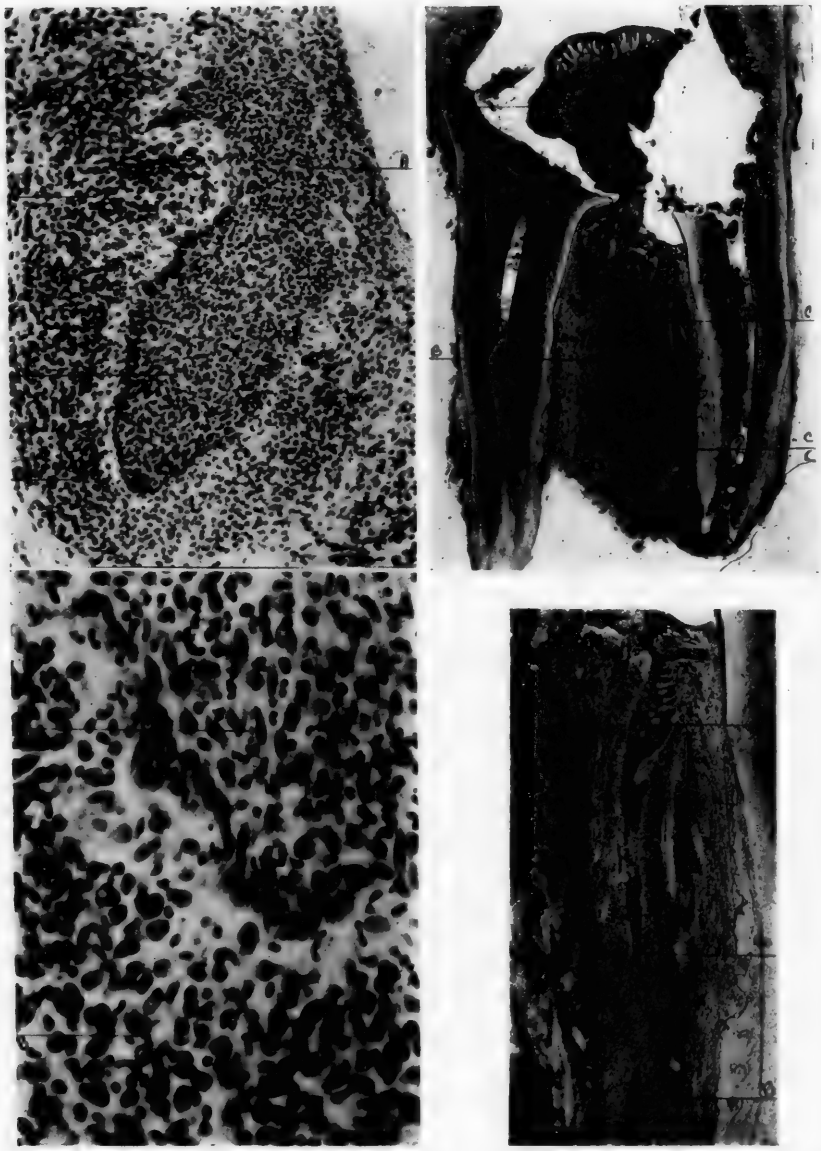

Fig. 1 (Upper left). Migration of leukocytes into epithelium (a) of gingiva. Subepithelial connective tissue (b) is infiltrated by plasma cells.

Fig. 2 (Lower left). Higher magnification of tip of epithelial peg from *fig. 1*. Leukocytes have accumulated in epithelium (a) while in connective tissue, plasma cells form infiltrate (b). Few leukocytes are present between plasma cells.

Fig. 3 (Upper right). Granulation tissue from bifurcation of molar extends into pulp chamber (a). Tissue between roots is densely infiltrated by plasma cells (b). Epithelial cords (c) connect apex of one root with tissue in pulp chamber.

Fig. 4 (Lower right). Higher magnification of inter-radicular granulation tissue from fig. 3. a, dense accumulation of plasma cells; b, epithelial cord infiltrated by leukocytes.

with plasma cells. Only the connective tissue which immediately surrounds the epithelium contains any leukocytes. In some sections the epithelium is so densely infiltrated with leukocytes that it can not be recognized, and its presence can only be surmised after a study of many sections.

There is a possibility that some toxins might penetrate between the intercellular spaces of the epithelium from the surface, extend far into the deeper tissues and, by positive chemotoxis, stimulate selectively the leukocytes to invade the epithelium in an effort to neutralize the toxins. Toxins might also be produced by a degeneration of the epithelial cells resulting from insufficient

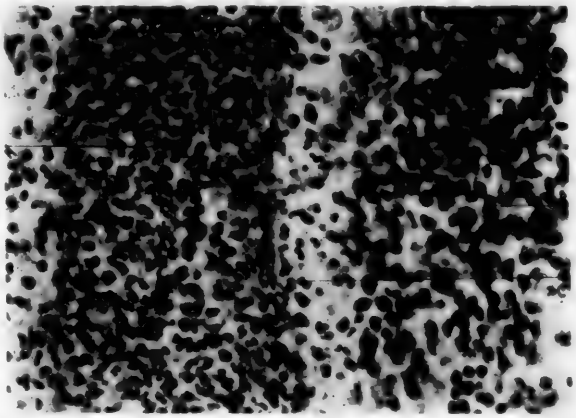

Fig. 5. Higher magnification of epithelial cord (b_1) from fig. 4. Epithelium is crowded with leukocytes (a). Surrounding connective tissue is infiltrated by plasma cells (b). Only zone immediately limiting epithelium contains leukocytes (c).

nourishment in the chronically inflamed environment. The partial destruction of the epithelial tissue might be the cause or the result of the invasion of the leukocytes.

CONCLUSIONS

Leukocytes often invade the epithelium of the surface, as well as the epithelium which has proliferated into the deeper regions, even if the surrounding connective tissue is in a state of chronic infiltration. This accumulation of leukocytes is most probably due to some chemotactic influences from toxins penetrating from the surface, or developing in the epithelium as a result of degeneration of the epithelial cells.

ANALYSIS OF THE ENAMEL FORMATION IN THE CONTINUOUSLY GROWING TEETH OF NORMAL AND VITAMIN C DEFICIENT GUINEA PIGS[1]

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The present study on amelogenesis is a histo-physiological analysis of the tissues involved in the formation and maturation of the enamel matrix. The mammalian tooth-germ or the successive phases of tooth development in a continuously growing tooth seemed to be especially suitable objects for this kind of investigation. Amelogenesis consists of growth, differentiation and production. These are the functions of the specific combination of tissues in the tooth-germ. While the morphological details are not neglected our interest centers about correlation and integration of various structures in the initiation, maintenance and termination of the complex process of amelogenesis.

MATERIAL AND METHODS

This study is based on more than 100 guinea pigs ranging from embryos to animals of about 1½ years of age.

The animals were kept on a basic diet which provided them with all the necessary food stuffs. The antiscorbutic factor was supplied by orange juice. In the experimental animals the antiscorbutic factor was either withheld or was added again when healing of the injuries due to the dietary deficiency was desired.

It is known that any general pathologic condition of the body may be reflected on growth and development of the teeth. The health of animals used for studies of the histogenesis of dental tissues must be kept under constant control. This requirement was met by recording the weight of each animal at regular intervals and by a complete autopsy including histologic sections of the organs in every case.

Although there is no difference between the dental tissues in the upper and the lower jaws, the lower jaw was given preference since it can be removed without injury of the periodontium. Fixation with Zenker's solution plus formalin or with Bouin's fluid was done either by immersion or by perfusion. No difference in the results has been found under the two ways of applying the fixatives. With any technic the preservation of the inner enamel epithelium, particularly its proper relation to the enamel matrix, is often found unsatisfactory. The epithelium is most vulnerable in the zone of transition between the tall prismatic and the reduced ameloblasts. This seems to be due to the quick change in

[1] Part of the expense of publication of this paper has been borne by the Walter G. Zoller Memorial Clinic. Received for publication, October 28, 1944.

hydration which has been found to take place during this period of development (see p. 485). The lower jaws were completely isolated and freed from the adherent soft tissues including the periosteum. In addition, the posterior part with the articular and muscular processes was removed. This helps to secure the best possible fixation of the dental tissues especially the inner enamel epithelium. Decalcification of the jaw in 5% nitric acid, imbedding in celloidin, cutting and staining was done in the usual manner. For demonstrating the presence of calcium in tissues the von Kossa method in McLean's and Bloom's modification was used. Fresh tissues were studied under various conditions in order to control the findings in the fixed and stained sections.

SURVEY OF THE DEVELOPMENT OF THE ENAMEL

The morphology of the continuously growing teeth of the guinea pig has been described by Gottlieb and Greiner ('23) and by Santoné ('35), among others. It suffices, therefore, to review briefly the structures as presented in the longitudinal section of the tooth (*fig. 7*). A single section through the upper or the lower jaw, as in *fig. 7*, does not show the full longitudinal section of a tooth in all its parts on account of the curved shape of the jaw and of the corresponding position of the teeth within the jaw. A schematic drawing (*fig. 1*) will be useful to demonstrate the 3 parts or lamellae of the tooth which are separated from each other by periodontal tissue. A diagrammatic section in *fig. 2* corresponding to a level between I and II in *fig. 1* simplifies visualization of the shape of these folded teeth with the periodontal tissue growing in from the buccal and the lingual side and the narrow "bridges" which connect the 3 lamellae. As the lamellae are identical, a central section of 1 lamella illustrates the complete formation of the enamel.

At the apical end of the tooth an enamel organ persists throughout life (I in *fig. 1*). Here the inner enamel epithelium (a) resting upon the young dentin (d), with odontoblasts (o) and the pulp (p) at the inner side, and stratum intermedium (st. i.), stellate reticulem (st. r.) and outer enamel epithelium at the outside are recognized. This is the embryonic zone of the tooth. It is also the sphere of growth with growth centers in the pulp, in the pseudostratified inner enamel epithelium and in the stratum intermedium. The rapid multiplication of the cells within the centers of growth has been studied recently by the aid of the colchicin method (Sicher '42, Jump '42).

In this region the tooth elongates continuously toward the surface. In particular the pulp tissue with the odontoblasts and the dentin as well as the ameloblasts and cells of the stratum intermedium are steadily moving upward (see arrows in *fig. 1*) while the outer enamel epithelium and the stellate reticulum are left behind. It is as though an ordinary mammalian tooth germ were opened at the top to allow the inner part with the hard substances to rise into the connective tissue.

In the cheek teeth of the guinea pig the connective tissue that fills the space between the lamellae is also part of the growing system. It moves up (arrow in *fig. 1*) with the tooth to which it becomes firmly attached. By a continuous

transformation into cartilage as a kind of crown cementum (c.c. in *fig. 1*) it becomes functionally adapted to the chewing surface where it undergoes attrition.

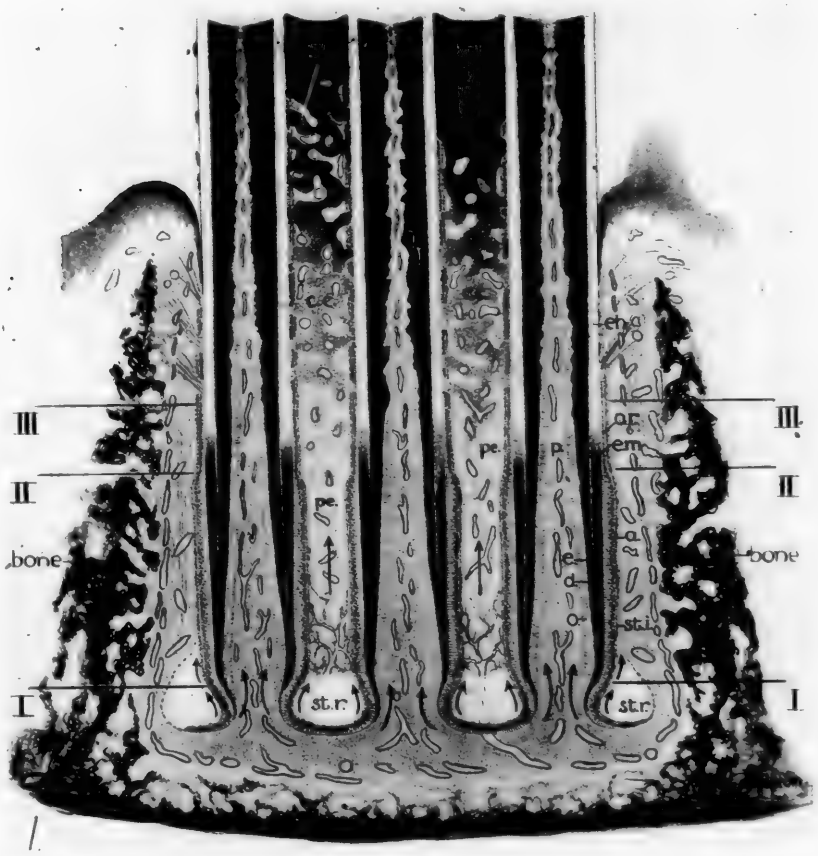

Fig. 1. Diagram of longitudinal section of guinea pig molar in alveolar socket. I, embryonic zone; I–II, zone of enamel production; II–III, zone of maturation of the enamel matrix. Three lamellae of tooth with pulp (p) and odontoblasts (o), dentin (d), enamel matrix (em) and hard enamel (eh), inner enamel epithelium with tall ameloblasts (a) during production, reduced ameloblasts (ar) during maturation and stratum intermedium (st. i.). In embryonic zone sellate reticulum (st.r.) and outer enamel epithelium. Lamellae are separated by the periodontal tissue (pe) which changes into cartilage cementum (c.c.) toward the occlusal end of the tooth. Periodontal tissue also between tooth and alveolar bone. Arrows indicate direction of growth in pulp, enamel epithelium plus stratum intermedium and in periodontal tissue.

Accordingly, the periodontal tissue regenerates from below by the steady expansion of a vascularized young connective tissue. Thus the growth process as a whole rests not only on the expansion of the pulp, the inner enamel epi-

thelium and stratum intermedium but also on the development of the embryonic periodontal tissue by multiplication of cells and formation of new blood capillaries. Pulp and periodontal tissue contain mesenchymal growth centers in contrast to the purely mitotic centers of the inner enamel epithelium and stratum intermedium. By this distinction we demonstrate a complexity of the growth process which helps to explain certain disturbances in the development of these teeth in scurvy (see p. 477).

It is necessary to relate the history and the activity of the ameloblasts to the continuous growth of the teeth. One after another, the ameloblasts are moving

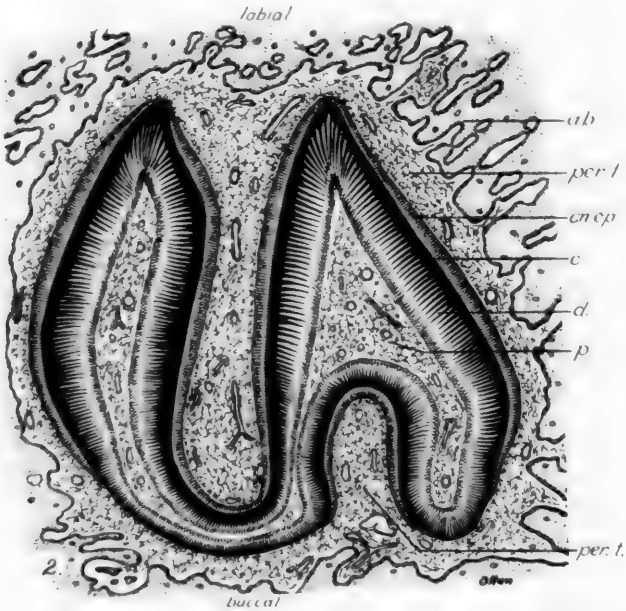

Fig. 2. Diagram of cross section of guinea pig molar at level between I and II of *fig. 1* a.b., alveolar bone; per.t., periodontal tissue; en.ep., inner enamel epithelium; e., enamel; d., dentin; p., pulp.

out of the embryonic zone. The same ameloblast, which emerges from the final mitosis within the embryonic zone in *fig. 1*, would be found a few days later higher up within the zone of production; still later, during the maturation period it is located in the short enamel epithelium; finally it is simply a cell in an epithelial islet adhering to the hard enamel. Both the cell and its product move toward the surface so that at any given level of the tooth the amount of enamel records, roughly, the activity of the cell up to that point. The cells of the stratum intermedium progress side by side with the ameloblasts.

The ameloblasts on their way toward the surface are subject to changing environmental situations. At their inner ends, the dentin increases, while at the outer side, the stellate reticulum is replaced by the young periodontal tissue, which

gradually turns into cartilage. In addition, the enamel substance, between the inner enamel epithelium and the dentin, is another environmental factor which changes from level to level in volume and physico-chemical constitution. It will be necessary to consider the influence of these environmental factors on the ameloblasts in the different periods of their history. *Fig. 1* shows the different zones of the tooth, viz. the embryonic zone, the zone or period of production of the enamel matrix (from I to II); the period of maturation (from II to III); and the zone of the hard enamel represented in decalcified sections by the enamel space with inconspicuous remnants of the finely distributed, scanty, organic material. The differences between these zones, with respect to the tissues involved in the production of the enamel, will be discussed below, giving special attention to the changes which characterize the transitions from zone to zone.

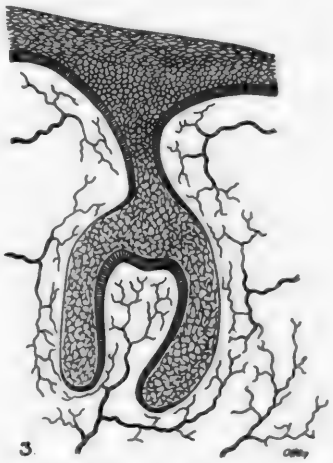

Fig. 3. Diagram of tooth germ and vascular mesenchyme to show 2 sources of supply, viz. pulp and surrounding tissue.

BEGINNING OF ENAMEL PRODUCTION

At the occlusal end of the embryonic zone, the whole system of tissues, which is the equivalent of the ordinary mammalian tooth germ, undergoes a series of changes. The differentiation of the embryonic cells of the inner enamel epithelium into active ameloblasts is but one part of a complex process. A histo-physiological analysis of this process shows that the ameloblasts are activated by changing metabolic conditions.

(1) *The metabolic conditions in the embryonic zone.*

The tissues of the enamel organ are supported by the pulp and are surrounded by the equally vascularized periodontal tissue (*fig. 3*). Obviously, it lies between 2 sources of nutrition. The pulp remains in its original relation to the epithelium as the supporting vascular mesenchyme; accordingly, the basement membrane of the mouth epithelium becomes the basement membrane of the inner enamel epithelium. The surrounding mesenchyme, on the other hand, envelops the tooth

germ and covers its surface. There the original basal layer of the stratified epithelium changes into the squamous outer enamel epithelium. In this way, a difference in conditions of material supply is established between the inner enamel epithelium and the rest of the tooth germ.

The immediate influence of the surrounding vascular system upon the enamel organ is indicated by the expansion of the intercellular spaces within the stellate reticulum, due to the uptake of water by way of the outer enamel epithelium, the limiting membrane. The evidence for this is provided by the microincineration studies of Hampp ('40). He found evidence not only of more Ca and Mg ash in the enamel organ than in the oral epithelium, but the surrounding connective tissue cells also appeared richer in ash than other fibroblasts. This indicates that the minerals stored within the stellate reticulum are taken in by the enamel organ by way of the outer enamel epithelium. The existence of a stratum intermedium which does not expand and of the well known strand of epithelial cells, which remains solid in the early stages of mammalian tooth germs, are further evidences that the swelling of the epithelium is due to an infiltration from the outside.[2]

While the bulk of the enamel organ is drawing fluid and dissolved material from the outside, the inner enamel epithelium and the stratum intermedium are still attached to the primary source of supply. Prior to the formation of dentin, the structural relation of the inner enamel epithelium and the dental papilla are unchanged. This fact implies the maintenance of the original functional relation. The high mitotic rate of embryonic ameloblasts and stratum intermedium cells indicates clearly their unchanged metabolic situation, in contrast to the cells of the stellate reticulum which are already engaged in collecting specific material to be used afterwards by the ameloblasts.

(2) *Beginning of amelogenesis.*

The initiation of amelogenesis is signalized by the concurrence of typical changes in the ameloblasts and in their relationships. They are: (1) the polarization of the ameloblasts; (2) the opening of a gap between the inner enamel epithelium and the already formed dentin; (3) the demarcation of the enamel epithelium from the stratum intermedium. *Fig. 9* shows the 3 modifications developing simultaneously within an area of transition between the embryonic zone and the zone of production. The hematoxylin-stained granules are the first enamel substances to be laid down at the surface of the dentin.

The polarization of the ameloblasts manifesting their differentiation into secretory cells does not need to be described in detail. The migration of the nuclei as far outside as the space allows is accompanied by the transfer of the Golgi material from the outer to the inner end of the cell (Jasswoin '24; Beams and King '33; Erausquin '39) and by the accumulation of specific granules at the cell base. *Figs. 9* and *11* indicate that the nuclei are gradually moving to the outer pole.

[2] For detailed observations on the stellate reticulum see Santoné ('35 b). This author stresses the enormous imbibition of water by the epithelium and explains the presence of the so-called enamel cord by a retarded imbibition.

Although the transformation of the pseudostratified enamel epithelium into a tall columnar epithelium has been often recorded, the problems presented by this change have not even been stated. The embryonic ameloblast is a spindle-shaped cell with the nucleus in the middle portion between the 2 filamentous ends which may be equal in length or more or less unequal depending on the position of the nuclei in the epithelium. Each ameloblast extends throughout the width of the epithelium. The transformation into a polarized cell leads to intracellular differentiation into a supranuclear, nuclear and infranuclear zones typical of secretory or absorbing cells. Such cells are functionally oriented toward a source of supply which in the case of a secretory activity lies at the infranuclear or basal pole of the cell. The conversion of the embryonic ameloblast into this type cell expresses, therefore, the functional orientation toward the stellate reticulum and the periodontal vascular system.

The statement made before that the ameloblast prior to its transformation is drawing fluid and nutritive material exclusively from the pulp may be an oversimplification. The shape of the embryonic ameloblast, differing as it does from the basal cell of a stratified squamous epithelium, suggests some early influence from the stellate reticulum. However, the cell is essentially indifferent. The polarization may be a gradual one, but it indicates a complete reversal in the supply of the ameloblasts. After this first turning point in the history of the ameloblast (in distinction from a second turning point later), the cell is drawing fluid and nutritional as well as specific materials exclusively from the outer side. The structural change goes hand in hand with the commencement of the specific activity as can be seen in *fig. 9*, where the first particles of enamel substance appear while the nuclei are still in migration. There is no better example in histogenesis to illustrate the parallelism of structural and functional differentiation. The ameloblasts also confirm the rule that the specific activity, as long as it remains on a certain level, renders the cell unable to divide (Wassermann '29, p. 495), for mitoses are completely absent as soon as the differentiation starts.

The acquisition of the new organization by the ameloblasts occurs at a definite level of the tooth. It has been suggested repeatedly that the differentiation of the ameloblasts may be stimulated by the so-called vascularization of the enamel organ (Gulat '36; Green '38). The ingrowth of blood capillaries into the enamel organ is, however, no regular feature of amelogenesis, as it varies greatly among different species and different types of teeth (Jump '38). Our case represents an extreme vascularization as the outer enamel epithelium and the stellate reticulum are replaced by a vascularized mesenchyme at the end of the embryonic zone. However, the transition from the embryonic to the active ameloblast takes place before the inner enamel epithelium plus stratum intermedium contacts the capillaries (*fig. 8*). Therefore, a better blood supply can hardly be the cause of the fundamental change within the ameloblast. The studies of Hampp definitely confirmed the idea that the stellate reticulum serves as a store house of materials necessary for amelogenesis. The ameloblasts utilize these substances faster than they can be replaced and begin to draw them directly from the blood stream when the stellate reticulum becomes exhausted. Thus, the

vascularization of the enamel organ seems to be rather the effect than the cause of amelogenesis. Increasing oxygen requirement may play its part in the change.

If the turning of the ameloblasts to the outer source of supply is not caused by materials advanced by approaching blood vessels, another factor must be sought which changes the environmental situation of the ameloblasts at the level where they begin to differentiate. In this respect, it is of special interest to remember that the formation of dentin regularly precedes the beginning of amelogenesis. No exception to this rule is known (Lehner and Plenk, p. 475). The remark recently made by Saunders, Nuckolls and Frisbie ('42) that occasionally the first enamel granules may appear prior to the formation of dentin is not substantiated by any available evidence. The chronological relation of dentin formation and initation of amelogenesis suggests a direct causal relationship. As a matter of fact, the activity of the odontoblasts alters the basement membrane which supports the enamel epithelium prior to the appearance of the earliest dentin. *Fig. 10* shows how the fibrils and the ground substance of the dentin develop in direct continuity with the basement membrane. The basement membrane, by its change into a layer of dentin, ceases to be a structure that serves the exchange of material between epithelium and connective tissue. It turns, on the contrary, into a barrier that separates the ameloblasts from the pulp and forces the ameloblasts to make use of the other available source of supply at the outside, viz. the stellate reticulum. The materials taken in from there initiate chemical processes which start at the base of the cell and continue toward the opposite pole, thereby producing a metabolic gradient which not only maintains the uptake of materials from the outside but necessitates an abundant influx of fluid. It shall be shown that the stratum intermedium cells are subject to the same changing condition, but, on account of their arrangement, in a somewhat different way.

The best evidence for the causal relation of dentin formation and beginning activity of the ameloblasts is derived from instances in which enamel and dentin formation are interrupted at the same level (*fig. 15*). In normal tooth development, the assumed coordination is strongly suggested by the coincidence of the first changes in the enamel epithelium with a definite density of the dentin (*fig. 11*). The youngest dentin shows a loose fibrillar structure containing so little ground substance that it stains only faintly blue in Azan preparations. It takes a dark blue color at a definite distance, the diameter of 15 to 20 odontoblasts, from the youngest odontoblasts. At this level (*a* in *fig. 11*), the first evidence of basal migration of the nuclei is apparent, and a space appears between enamel and dentin. These conditions strongly suggest a separation of the ameloblasts from the original source of supply and an inflow from the opposite direction.

This analysis is compatible with the findings of Huggins, McCarroll and Dahlberg ('34) in their studies on the experimental heterotopic formation of dentin and enamel. The soft tissues of the developing permanent canine tooth of young dogs were transplanted into the abdominal wall of the animals in various combinations. Only when the odontoblast pulp membrane and the embryonic portion of the epithelial layer were transplanted together so that they stayed in contact,

were dentin and enamel formed in their regular positional relationship. Pulp tissue with odontoblasts, alone, produced a more or less irregular dentin. The enamel epithelium, without odontoblasts or out of contact with odontoblasts, failed to persist as prismatic epithelium or to produce enamel. In the 6 identical experiments, which resulted in "the preservation of the cylindrical character of the ameloblasts and the formation of new enamel," the latter was found "in normal relationship to these cells and the newly formed dentin." Huggins, McCarroll and Dahlberg came to the conclusion that "the influence of mesodermic connective tissue derivatives on the form and function of epithelium" has been demonstrated by these experiments. I believe this influence can be understood on the ground of the normal histogenetic relationship of the enamel epithelium to odontoblasts and dentin. Prior to the formation of dentin, the epithelium is attached to the odontoblasts by the above demonstrated basement membrane. The preservation of the character of the epithelium is due to this attachment. The polarization of the ameloblasts occurred in the transplants only when they were in contact with the dentin. Their separation results in the degeneration of the epithelium without enamel formation. These findings point directly to the dentin as the decisive factor in enamel formation.

The space which arises between enamel epithelium and dentin (*figs. 8, 9 and 11*) at the critical time is a necessary corollary of amelogenesis. It remains open throughout the period of enamel production and disappears at its conclusion (*figs. 23* and *24*). That is to say when the enamel formation begins it becomes the space between epithelium and enamel matrix (*figs. 21* and *23*). *Fig. 23* illustrates this situation as reported by numerous previous investigators. Although the split is often enlarged by fixation and decalcification, there is no reason to doubt its existence in the living tooth. It is found in sections of both decalcified and undecalcified specimens. What is more important, by isolating the enamel epithelium from fresh teeth, a layer of vacuoles is sometimes found adhering to the secretory poles of the ameloblasts. These vacuoles can be considered as the equivalent of the space we are describing here for reasons given below. The phenomenon is introduced as "space" between enamel epithelium and dentin, or enamel matrix and dentin, in order to direct attention to the mechanical factors responsible for the receding of the ameloblasts from the tissue to which they were closely attached in the embryonic part of the tooth. The situation presented in *fig 9* can be explained best by an emission of fluid at the secretory pole of the ameloblasts under a certain pressure and in such an amount that it cannot be absorbed immediately by the enamel matrix. Furthermore, the opening of the space is clearly indicative of the previously mentioned reversal of the flow of fluid to the ameloblasts at the time they become separated from the pulp by a layer of dentin. It is clear that the space is not simply filled by a fluid secretion, but contains a more or less highly vacuolated material (*fig. 21*). The emission of fluid is, therefore, associated with a second process, that is the production at the secretory pole of the ameloblasts of a material which holds the fluid in its vacuoles see p. 479).

Previously, the development of enamel has been described mostly in static

terms and without reference to the processes which create and maintain or change the structures. The discussions, so far as this particular space is concerned, were mostly confined to consideration of structural details such as the Tomes' processes and the ameloblastic membrane, without deciding between controversial interpretations. The terminal bars (*fig. 21* and *26*) are the only structures of special interest in this part of our analysis. In the enamel epithelium, as in any other prismatic epithelium, the material that fills the intercellular spaces at the secretory poles of the cells and appears as terminal bars in the sections marks the border or the surface of the cells. This point was neglected by Held ('26) when he described the enamel formation as an entirely intracellular process. Recently Saunders, Nuckolls and Frisbie arrived at a similar interpretation, describing the vacuoles (*fig. 21*) as intracellular formations. These authors did not overlook the terminal bars but eliminated them from the discussion as artifacts due to decalcification. However, the presence at the inner ends of the ameloblasts, in vivo, of a cementing substance, which in sections appears as terminal bars, can be demonstrated beyond doubt by a simple experiment. When isolated fresh enamel epithelium is treated with 30% alcohol, the macerating agent does not at first entirely separate the cells from each other. The internal ends remain attached and isolated groups of ameloblasts are found with the outer ends spread while the inner ends are kept together by the cementing material. As far as the distinction between intra- and extracellular structures at the surface of a cell is possible, the vacuolated material between enamel epithelium and matrix in *fig. 21* is certainly extracellular as it lies beyond the terminal bars. It may be added that the vacuoles, as shown in *fig. 21*, are by no means so regularly arranged as to warrant the statement by Saunders, Nuckolls and Frisbie that each cell contains a single vacuole within the Tomes' process.[3]

The opening of the space between enamel epithelium and dentin, which coincides with the other signs of beginning activity of the ameloblasts, is to be explained by a free passage of fluid through the cell. This interpretation has been shown to be correct by the aid of vital staining.

Ganzer, as early as 1906, in a remarkable article on physiologic injections, reported a flow of the staining material from the ameloblasts into the secreted product. More recently, Blotevogel ('23) found the active ameloblasts in the tooth germ of the mouse stained with trypan blue and the enamel matrix became infiltrated by the dye.

A single injection, either subcutaneously or intraperitoneally, of 10 cc. of a 0.5% aqueous solution of trypan blue into guinea pigs weighing about 300 grams

[3] Orban, Sicher and Weinmann ('43) are of the opinion that the vacuoles between the Tomes' processes are artifacts found only "in specimens not so well fixed." It was pointed out here that the aspect of these vacuoles may vary depending on the intensity of transfer of fluid through the ameloblasts. *Fig. 32* shows the vacuoles somewhat enlarged at 2 places in the section. This is probably due to fixation. But between these 2 places the space between epithelium and matrix as well as the vacuoles are of usual size, according to my experience. The most dependable criterion whether or not a structure is an artifact is offered by the observation of fresh tissue whenever such control is possible. In regard to the vacuoles this test is affirmative as was pointed out on page 471.

produced, in general, the same results as obtained by the previous authors. A general histologic survey of all the injected animals makes it possible to say that the odontoblasts and the ameloblasts, together with certain of the connective tissue macrophages, are the first cells of the body to store trypan blue in the form of fine granules. It is still more important to note that in the continuously growing teeth the embryonic ameloblasts do not take up the dye. The capacity for storage begins at the level where evidence of secretion is first noted (*fig. 12*). The beginning of vital staining at the level where all the other signs indicate the turning point in the history of the ameloblasts is a direct demonstration of the intensity of absorption, particularly of water, by the ameloblasts. The transfer of material here is so great that some of the dye is segregated in the cytoplasm, while the rest passes into the enamel matrix. Considering the whole syndrome of signs that indicate the turning of the ameloblasts to the outer source of supply, it cannot be doubted that the trypan blue is taken from the outside. The ameloblasts do not stain vitally as long as they are supplied by the pulp although the dye is present at their inner ends in the odontoblasts and even within the basement membrane. Apparently the latter does not allow the dye to diffuse into the ameloblasts and they do not take up the dye until their functional polarity is established.

It is of special interest that the stratum intermedium cells do not react to vital staining as do the ameloblasts. They may contain trypan blue granules but although they are able to absorb the dye, as a rule they do not store and precipitate it. Other substances, as we shall see later, likewise are passed on by the stratum intermedium cells to the ameloblasts. Under certain conditions and regularly in the beginning and at the end of enamel production, the globular material (see p. 476), like the dye, is retained within the stratum cells. The findings of vital staining in the stratum cells, therefore, parallel the findings concerning the material which is usually passed on to the ameloblasts as soon as it is prepared by the stratum intermedium cells. Accumulation of such substances indicates a slowing down of the flow of fluid toward the ameloblasts as is certain to occur at the end of amelogenesis under normal circumstances. A slight retardation in transferring the material may be enough to cause concentration and precipitation of trypan blue within the stratum intermedium cells.

The demarcation between enamel epithelium and stratum intermedium is no less significant than the other changes which occur simultaneously within the area shown in *fig 9*. Mummery ('24, p. 117) describes it in these words: "Before the commencement of calcification (amelogenesis in Mummery's terminology) many cells of the stratum intermedium are seen lying close to and often between the distal ends of the ameloblasts, but after calcification has commenced they are completely shut off from these cells by a membrane, the outer ameloblastic membrane of Williams." The existence of this membrane has often been denied for 2 reasons, so far as I can see. First, the base of the enamel epithelium is not always a sharp line; secondly, the ameloblasts and stratum intermedium cells are connected with one another by protoplasmic processes. On the other hand, the striking difference in their relation during the period of production and

during the period of maturation cannot be neglected (*figs. 23* and *24*). Indeed, it is more conspicuous than the corresponding difference between embryonic and secretory period. *Figs. 26* and *27* show in detail the separation of stratum intermedium cells and ameloblasts in the production phase and their intimate association in later phases; this is true even though the cell types are connected by protoplasmic processes in both phases. There can be no doubt that the demarcation between enamel epithelium and stratum intermedium is as necessary a corollary of amelogenesis as the other structural characteristics already discussed. It indicates the establishment of an absorbing surface at the time when the ameloblasts begin to draw material from the outer side. Like any other continuous protoplasmic surface layer which performs membrane functions, this line of demarcation is unstable. It may disappear and reappear in response to changing conditions. It is, therefore, not surprising that the demarcation is occasionally missing along a few ameloblasts. The existence of the membrane as a physiological, rather than as an anatomical, membrane cannot be denied. It is part of a continuous surface layer which covers not only the outer ends of the ameloblasts, but also the cell processes and continues into the membranes of the stratum cells.

The absorbing surface at the one hand and the direct protoplasmic connection with the stratum cells on the other represent 2 ways of supply for the ameloblasts: one by absorption through the outer ameloblastic membrane, another by direct transfer of material from the stratum intermedium cells. Fluids and dissolved materials are likely to be absorbed directly from the tissue fluid while materials which may be elaborated by the stratum cells will pass across the cell bridges. The supply of the ameloblasts by these 2 ways makes them different from any other secreting cell and indicates the close collaboration of ameloblasts and stratum cells.

(3) *Coincidence of interruption of dentin and enamel formation.*

The disturbances in the formation of dentin in scurvy come to a climax with the transformation of the odontoblasts into osteoblast-like cells the activity of which, however, is frustrated by the absence of ascorbic acid from the tissues (Wolbach and Howe '26). When this happens a layer of more or less defective dentin has already been deposited in the majority of cases. Consequently, the enamel epithelium borders on a dentin which, though defective, still plays its part in amelogenesis as a barrier between ameloblasts and pulp. The situation is quite different at places where the odontoblasts have been missing from the beginning and are replaced by non-polarized cells. This results in a complete interruption of the formation of dentin. *Fig. 15* illustrates such an event. Within a small area no dentin was laid down at the time when the ameloblasts emerged from the embryonic zone of the tooth. This was not due to a local disturbance, but to a general aggravation of the disease at this particular time since the same defect is found at the same level in all the teeth of the jaw. Above and below the defect the ordinary scorbutic dentin is seen. Within the area only a few fibrils and very little ground substance have been produced by the irregularly shaped mesenchyme cells. The most striking fact presented here is the

total absence of enamel exactly opposite the gap in the dentin. It is evident that the enamel epithelium was not put into action but maintained its embryonic character. Within the same area, where the ameloblasts did not become polarized, no space has opened toward the layer of fibrils which sustain the epithelium like an ordinary basement membrane. Furthermore, there is no demarcation between ameloblasts and stratum intermedium cells. In short, all the changes which are correlated with the commencement of amelogenesis are absent. This indicates, once more, that they constitute a syndrome which appears either in toto or not at all, depending on the presence or the absence of the one casual factor common to all those changes.

The idea that both odontoblasts and ameloblasts had been prevented from differentiating simultaneously by an unknown factor can be discarded for the following reason. It is well established that vitamin C deficiency always impairs the odontoblasts and has no direct effect on the ameloblasts (Boyle '38). The present work shows that it does not interfere with their differentiation (*fig. 19*). There is, therefore, no reason to doubt that in the case of *fig. 15* the suppression of dentin formation was the primary disturbance in tooth development and was responsible for the failure of the ameloblasts to differentiate. Thus, the total absence of both dentin and enamel at the same place and at the same time in development is further evidence for the direct causal relationship between dentin formation and initiation of amelogenesis, which has been suggested by analysis of the normal tooth development.

Although the presence of dentin opposite the enamel epithelium appears to be indispensable for activating the ameloblasts, it is certain that other local factors beside the dentin are equally important. This is suggested by the localized degeneration of the enamel epithelium in the early development of certain types of teeth. Examples of this kind are the rodent incisors in which the enamel is missing at the lingual surface, or the guinea pig molars with enamel free zones at the lingual and buccal sides over areas where there is a typical dentin formation. In these instances the regressive changes seem to occur when the enamel epithelium is still in the embryonic stage and before any differentiation has taken place.

THE BUILDING UP OF THE ENAMEL MATRIX

The material which eventually turns into the hard enamel is laid down in definitive thickness during the period of production. It has been called the enamel matrix or the preenamel and corresponds to M. Mellanby's ('29) first stage of the developing enamel. It consists of easily cut, stainable material. The surface of the enamel matrix opposite the ameloblasts presents the so-called honey-comb structure (*fig. 26*) as long as new material is being deposited. Other evidences of the pattern of the young enamel are rendered invisible by its intense adsorption of stains (*figs. 16* and *24*). When an artificial break occurs in the material, a fibrous structure becomes visible at the edges of the break. At the end of the period of production, the latent pattern becomes visible at the same rate as the stainability decreases (*fig. 31*).

In reviewing the first signs of amelogenesis (*fig. 9*), 2 processes involved in the

building up of the matrix are noticed, namely: (1) the production of a granular material with the same staining properties as the matrix, and (2) the elaboration of a framework filling the space between the ameloblasts and the dentin. These 2 processes and their close correlation will be the subject of the following discussion.

(1) *The secretion of the globular material.*

The material, which in the very beginning of amelogenesis appears as fine granules at the prospective dentino-enamel junction (*fig. 9*), is also found regularly within a few cells of the stratum intermedium in the form of larger droplets (*fig. 11*). This indicates that the stratum cells participate in its elaboration, for the droplets cannot have been moved from the ameloblasts to the stratum intermedium cells since at this time fluid is passing in the opposite direction as shown by vital staining (see p. 473). Material, which has the same staining property, is present at the secretory pole of the ameloblasts and is incorporated into the enamal matrix. Although it is not usually intracellular, it is sometimes present within both ameloblasts and stratum intermedium cells in regions which are producing normal enamel. Occasionally, ameloblasts are found which in the supranuclear part contain many more globules than appear in the ameloblasts of *fig. 16*. Whether or not the material is temporarily deposited within the cells depends on the more or less perfect equilibrium of elaboration and emission. This is made clear by the regular appearance of the same droplets at the end of production when the flow of material through the ameloblasts is slowing down to become finally arrested (p. 485).

The idea suggested by Welikanowa ('28), that the stratum intermedium cells, alone, are engaged in the secretion of this part of the enamel matrix while the ameloblasts would only transmit it to the outside, is not supported by any evidence. On the contrary, in cases of a disorganized enamel epithelium, sometimes found in scorbutic animals, the 2 kinds of cells, ameloblasts and stratum intermedium cells alike, contain the secreted material in comparatively large amounts, although they are no longer in their proper anatomic and functional relation. Evidently both types of cells continue to produce the substance when there is no longer a flow of material in one direction.

The conspicuous droplets within the ameloblasts have been known since the early studies on amelogenesis (Ancel, 1882; von Spee 1887) and have been called globular bodies. They are not always spherical, particularly not outside the ameloblasts where their irregular shape suggests a viscous material (*fig. 17*). According to Chase ('27, '29, '35) and to Saunders, Nuckolls and Frisbie ('40), the globular bodies take the same stains as keratin. Although a keratin was the only protein found in the hard enamel by Rosebury ('35), the globular bodies and the enamel matrix may well contain other proteins beside a keratin, as most of the organic substances are eliminated when the enamel matrix is transformed into hard enamel. As long as we do not know more about the chemical composition of the material in question, the indifferent terms, globular bodies and globular material, should not be replaced by specific terms such as hyalin or eleidin globules suggested by Saunders, Nuckolls and Frisbie.

The hydrodynamic factor in the secretory function has been demonstrated in the ameloblasts during the enamel production by vital staining (p. 473). That the globular material is eliminated by the cells under some pressure becomes particularly evident in the beginning of amelogenesis when the first particles are laid down within the dentin rather than upon it (*fig. 9*). In scorbutic teeth, the globular material may be found within the dentin and even among the odontoblasts (*fig. 18*). This is, of course, due to the abnormal consistency of the dentin, but is, nevertheless, again indicative of a considerable hydrodynamic pressure by which the globular material is swept on.

(2) *Production of the ground substance.*

Following the point of view already established, that the secretion of globular material is but one part of the process, we propose to demonstrate the production of globular material in the absence of the framework into which it is incorporated under normal conditions. This departure from the normal development occurs not infrequently when growth and eruption of continuously growing teeth are disturbed. Particularly in scorbutic animals, the mitotic and the mesenchymal growth centers of the teeth (see p. 466) may become functionally disrupted. The enamel epithelium, which is still growing but is prevented from moving up in the regular way, becomes folded (*fig. 18*). The secretion of globular material goes on but the product accumulates within the fold where the framework between and surface of the enamel matrix was mechanically destroyed. The additionally secreted masses of globular material are often rounded up, sometimes flattened toward the surface of the matrix, and differ from the enamel matrix in their homogeneity. In other words, secretion of globular material, without subsequent incorporation into a framework, does not lead to the formation of enamel. No attention has been given hitherto to the distinction between the formation of the enamel matrix as a complex process and the production of globular material alone. Otherwise the latter would not have been mistaken for a hypertrophy of the enamel by Diamond and Weinmann ('40), for instance, in their description of this same phenomenon in human tooth germs (their figs. 42–46). Since no additional enamel matrix is built up in these cases, the term hypertrophy is out of place and misleading for the nature of the disturbance is hypersecretion of globular material without the formation of enamel matrix.

A disturbance in tooth development, which is in sharp contrast to the hypersecretion of globular material, is presented in *figs. 19* and *20*. The conspicuous defect in this case, the only one of its kind in my material, is due to the absence of globular material from the ground substance. It may be called a formation of the ground substance without incorporation of globular material. The enamel formation is by no means completely interrupted as in the case of *fig. 15*. The formation of the dentin is not seriously disturbed and the enamel epithelium is in the proper state of differentiation. The space between dentin and epithelium is also open but is not empty. Under high magnification (*fig. 20*) its structure becomes apparent. Close to the ameloblasts there are the same processes as can be seen in normal material as Tomes' processes (*fig. 21*). They are separated from each other by the vacuoles described above. The Tomes' processes in

fig. 20 continue into a layer of fibrous substance which is well delineated from the dentin. The borderline between this layer and the dentin continues into the dentino-enamel junction at either side of the defective area. Most of the globules are retained within the ameloblasts or at their surface but at the right side of the photograph a few globules appear in their proper place. The explanation of this situation is simple and can be given without restriction. The framework normally covered by the globular substance, or in other words the structural part of the enamel matrix, was formed but remained empty as the globular material did not enter it. Again there is no enamel matrix built up.

In this case, the framework of the enamel matrix is certainly not the same as in the normal when it is impregnated with the globular material. It will contain less water, since the passage of fluid through the ameloblasts was evidently too slow to carry the globules to the surface, and accordingly the framework covers less space than under normal circumstances. Otherwise, the ground substance without globular material is not likely to be fundamentally different from that enclosed in the normal enamel matrix. The phenomenon shown in *figs. 19* and *20* is important since it offers the unique opportunity of studying the pattern of the ground substance which normally is obliterated by the globular material. The following remarks on the structure of the enamel matrix are based not only on the one illustrated but on the findings at corresponding levels in all the teeth of this jaw.

Under normal circumstances only the parts of the Tomes' processes next to the ameloblasts can be seen (*fig. 21*). These parts can readily be identified in *fig. 20*. According to the generally accepted idea, the Tomes' processes are supposed to continue as cylindrical units of the enamel matrix to the dentino-enamel junction. *Fig. 20* shows a quite different situation. The processes are spreading into the fan-shaped areas of the ground substance. These areas, in the section, correspond to conical parts which are, however, not sharply outlined and appear to overlap each other or to fuse with one another. The substance presents a fibrillous texture and it attached to the dentin in a minutely serrated juncture. It is of great interest that the same spreading of the Tomes' processes into the forming enamel of the kangaroo was described by Mummery ('24). This author said in the legend to his figs. 84 and 85 (tooth development of Macropus) in his textbook: "that at the honeycomb region the fibers of the Tomes' processes spread out in a fan shape from neighboring processes crossing one another and intermingling to be again drawn together to form the columns of the prismatic substance of the enamel." Mummery's and my own findings are identical but for his statement that the "fibers" are again drawn together. However, no evidence for this was given by Mummery. Although, in this way he attempted to reconcile his findings with the classic concept that the enamel prisms are from the beginning of enamel formation represented by structural units of the matrix, he contradicted this idea when he declared, "that the ultimate bundles of fibers which form the basis of the enamel prisms do not appear to be the direct prolongations of one particular ameloblast but are contributed to by those in contact within it on either side." The problem of the relationship between the primary struc-

ture of the matrix and the definite structure of the enamel has to be left to further investigation since our present knowledge is not sufficient to justify a statement such as that quoted from Mummery. The only conclusion which is strongly suggested by both Mummery's and my own findings is that the ultimate units of the enamel that are the prisms "do not appear to be the direct prolongations of one particular ameloblast."

A further approach to the problem of the primary structure of the enamel matrix can be made from the histogenetic angle. The material, which in the beginning of amelogenesis appears within the space between enamel epithelium and dentin (*fig. 22*), is the first deposit of the ground substance. It develops directly into the matrix and is infiltered slowly by the globular material. The scarcity of the latter in the beginning makes the situation similar to that of the pathologic case described in p. 478. There is no reason why the fundamental structure should not be recognizable at this early stage of development. However, the difficulty of interpreting the minute structures within the ground substance (*fig. 22*) is increased by their feeble staining. In addition, the details of the early ground substance vary considerably, depending on the variable effect of fixation. It is, therefore, not surprising that Prenant's (1924) description of these structures is in many details at variance with that of Studnička (1917). Although the finest structural details cannot be made out with certainty at the early stage of enamel formation, the examination of the material seen between enamel epithelium and dentin in *fig. 22* can be very helpful if it is correlated with the findings on the ground substance in the absence of globular material (*fig. 20*). In other words, the essential question is whether or not the early ground substance, as long as it has little incorporated globular material, reveals the same characteristics as presented by the ground substance without globular material. In *fig. 22*, where the terminal bars are already present, the Tomes' processes which are more or less distinctly related to a particular cell can be recognized. The vacuoles seen between them are identical with the vacuoles in *figs. 21* and *26*. Toward the dentin, the substance which surrounds the vacuoles and is continuous with the Tomes' processes fuses into a layer that covers the surface of the dentin. It must be admitted that the picture is not easy to understand at all places, insofar as it is sometimes uncertain whether a particular vacuole lies within the Tomes' process or between 2 neighboring processes. In many instances, however, the situation is the same as it appears later when the vacuolization has become stabilized (*fig. 23* or *26*) and the vacuoles can be seen clearly between the processes. The fusing of the supravacuolar parts of the ground substance toward the dentin is the same phenomenon which could be described as the spreading of the processes into fan shaped areas in the ground substance without globular material, for here the Tomes' processes are more distinct and the fibrillous structure is clear. Thus, the examination of the early ground substance corroborates the statement that the Tomes' processes do not exist as separate units throughout the ground substance or the matrix, respectively.

In *fig. 22* from the right to the left side the ground substance is already growing prior to the infiltration by the globular material of which only a few granules are

deposited at the prospective dentino-enamel junction. There cannot be any doubt that the ground substance will continue to grow further in the same way by a continuous formation of the Tomes' processes at the inner ends of the ameloblasts while the older parts of the processes are incorporated into the ground substance. The continual emission of fluid, which is slowly absorbed by the enamel matrix, maintains the vacuoles which however, may vary in size depending on the intensity of this particular phase of enamel production. The schematic drawings of *fig. 4* illustrate the growth of the ground substance as

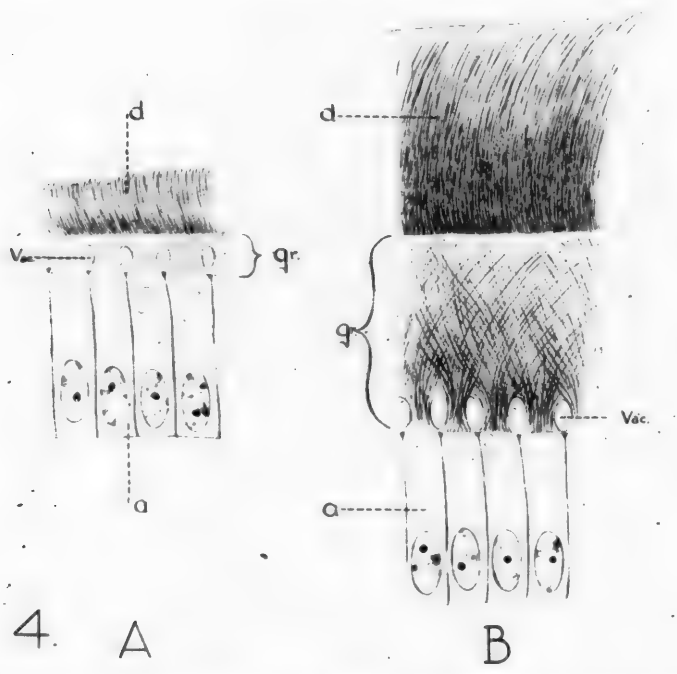

Fig. 4. Diagram to illustrate growth of ground substance (gr) of enamel matrix. A, ground substance at an early stage as in *fig. 22*, B, at a later stage as in *fig. 20*. d., dentin; vac., vacuoles; a., ameloblasts.

suggested by these findings. A, shows the framework of the enamel matrix at an earlier and B, at a later stage of development as it would appear if the globular material could be removed. This stresses the fact that, beside the secretory activity of the ameloblasts, a specific process of growth is involved in the formation of the enamel matrix. The primary pattern of the ground substance as suggested in *fig. 4* is changed at an early time when, beginning at the dentino-enamel junction, the formation of the prisms commences while at the opposite side the matrix continues to grow. As the formation of the prisms was not included in this study, *fig. 4* was left incomplete regarding this point. Mummery's

suggestion that the fibers from neighboring processes are again drawn together to form the prismatic substance of the enamel in kangaroos is not supported by present knowledge from other species. The relation between the primary structure of the enamel matrix and the definitive structure of the enamel is still the most difficult problem in amelogenesis.

The finding that the supravacuolar part of the ground substance in *fig. 22* covers the dentin as a continuous layer was anticipated by Studnička ('17). The fusing of fibrils arising from the Tomes' processes, as he described the phenomenon, convinced Studnička of the existence of a membrane identical with Huxley's "membrana preformativa substantiae adamantinae". He declared that enamel formation begins from this membrane and not from the bodies of the ameloblasts. The statement that enamel formation "begins" with the infiltration of the membrana formativa by the stainable material is not compatible with the results of the present analysis since it is now known that the formation of the ground substance to which such a membrane belongs is no less a part of the enamel formation than the secretion and emission of the globular material. Studnicka's opinion is however correct, insofar as the first enamel granules are deposited in the part of the ground substance that covers the dentin. Whether or not this layer of feebly stained material is continuous and, therefore, a membrane cannot be decided by studying sections which show the membrane in profile. Tangential sections, where the dentino-enamel surface can be brought into focus from above, offer a better opportunity at the time when enough stainable material has been laid down to make the dentino-enamel plane visible (*fig. 25*). Under these conditions a network of globular material, not a continuous layer, is found. This suggests a corresponding attachment of the ground substance to the dentin in the pattern of a network rather than as a continuous membrane. The pattern in which the globular material is laid down upon the dentin has not yet been given the attention it deserves. The arrangement of the first enamel granules in clusters may well be the first step in the formation of the prisms.

What kind of cellular process the formation of the ground substance is remains a question. There is no doubt that the ground substance is a product of the ameloblasts. However, it is not produced by secretion, like the globular material. No signs of secretion, such as granules or the expulsion of material, which might be related to the formation of the ground substance can be found. The product itself is a material of a characteristic structure and remains connected with the cells until the last of it has been produced. This suggests that the formation of the enamel ground substance is due to the same kind of productive cellular activity that is responsible for the elaboration of ground substances in general, e.g., by chondroblasts or osteoblasts, or of cuticles at the surface of epithelia. According to the general point of view held by Studnička ('29), Wassermann ('29, p. 612), Jasswoin ('31) and others, cell products of this kind result from the transformation of the outer part of the cell body into a material which is at first continuous with the cytoplasm but becomes more or less independent from the cell. To look upon the formation of the ground substance from

this general point of view does not mean an unqualified subscription either to the idea that the enamel is simpiy a kind of epithelial cuticle or to the so-called transformation theory.

The enamel as a whole is the product of both secretion of globular material and formation of a ground substance, but only the latter corresponds to an epithelial cuticle. The objection, for example by Held ('26), that a cuticle cannot be produced at the base of an epithelial cell shows only how completely the inversion of polarity of the ameloblasts at the beginning of amelogenesis has been neglected. When the ameloblasts turn to the outer source of supply, as has been described in this paper, the former base of the cell changes into a free surface which as such is the site of both secretion and formation of a cuticle-like material.

The transformation theory interprets the formation of the enamel matrix as a direct transformation of the ameloblasts into the enamel rods until at the end of enamel production a reduced cell remains. The term hyalinization used in connection with the transformation by Saunders, Nuckolls and Frisbie indicates that the cytoplasm of the ameloblasts is supposed to change into a homogeneous material of structureless protein substances. These authors who are the most recent to revive the transformation theory illustrate their concept of amelogenesis by a schematic drawing which shows the ameloblasts decreasing in height at the same measure as the enamel rods are growing in length. In this form, the theory is not compatible with the findings reported in this paper. The elaboration of a ground substance, which is one part of the enamel formation, is by no means a using up and shrinking of the cytoplasm. It is a process of production, that is of cellular activity, accompanied, as a rule, by compensatory growth of the living matter which keeps the structure and function of the cell more or less completely intact. That this is true of the ameloblasts is shown by the camera lucida drawing of the entire length of a random sample of the enamel epithelium illustrating the stages from early activity to the period of maturation (*fig. 5*). Throughout the production of the enamel matrix, there is no decrease in the height of the ameloblasts.[4] The reduction of the cells occurs at the end of this period when all the enamel matrix has been formed. Thus, the reduction of the ameloblasts has nothing to do with a tranformation as suggested by the theory. Indeed no such transformation occurs. When a slight decrease of the ameloblasts is found during the production period, as often happens, it is far from being proportional to the mass of enamel produced. Finally, the most important argument against the transformation theory is the fact derived from findings of Chase and of the present author that the ameloblasts, after their productive activity, though reduced in size are still exerting an indispensable function in the process of maturation of the enamel matrix (see p. 488).

Of the recent studies on amelogenesis which took the formation of the enamel matrix into special consideration, Studnička's work came closest to the results presented in this paper. Studnička had distinguished clearly between secretion and formation of an exoplasmic ground substance as the 2 main pro-

[4] The same statement has been made recently in Orban's textbook on oral histology and embryology ('44, p. 87 and 91).

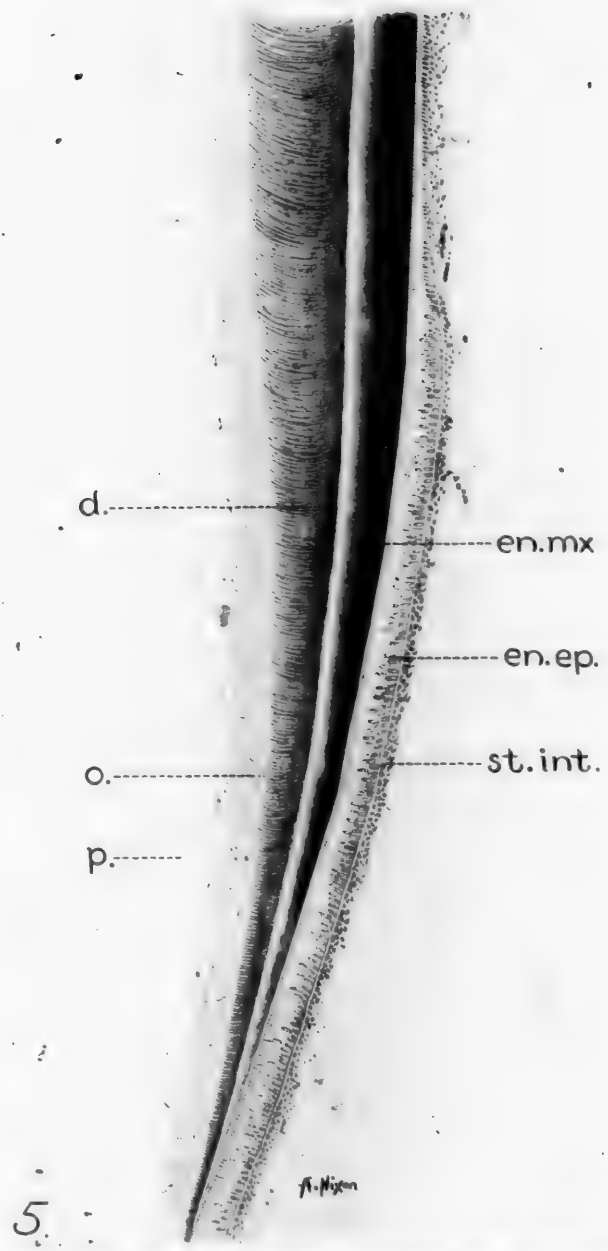

Fig. 5. Camera lucida drawing of enamel epithelium from beginning of activity of ameloblasts to period of maturation of enamel to show that ameloblasts do not decrease in height until all enamel matrix has been formed. Reduction of tall ameloblasts marks end of production and beginning of maturation of enamel matrix. en ep., enamel epithelium; st.int., stratum intermedium; d., dentin. Drawn with Zeiss apochr. AA, oc. 10× at table level.

cesses involved. Weidenreich, in his paper on the enamel and its relation to the dentin ('26), thought it most probable that the organic "Grundmasse" is the product of a secretion by the ameloblasts. Jasswoin ('31) agreed in general with Studnička and me as to the nature of ground substances. However, in his work on amelogenesis ('24) he considered only the interprismatic substance of the enamel as a ground substance. To indicate that considerable differences exist between Jasswoin's description of the enamel formation and the results of the present work, it may suffice to quote Jasswoin's statement that the granules produced by the ameloblasts "move along the Tomes' processes and appear to overflow the latter, fusing into a uniform mass."

THE END OF PRODUCTION AND THE MATURATION OF THE ENAMEL MATRIX

The period of production comes to an end when the enamel matrix has attained the definitive thickness of the hard enamel. However, this is no absolute measure as the enamel tapers off toward the enamel free areas of the tooth. From the causal point of view, it seems to be more important that the production should stop at the same level throughout the tooth and, moreover, at the same level in all teeth of normal growth. This points to a change in the environmental conditions of the ameloblasts as the causal factor for the termination of enamel production. The opposite suggestion of a limited life span inherent to the ameloblasts (Stein '34), although widely accepted, can be discarded. When the enamel epithelium becomes folded up in consequence of a disturbance of growth and eruption (*fig. 18*), much more enamel material may be produced over a longer period than the same ameloblasts would have produced under normal circumstances. Furthermore, it will be shown that the ameloblasts are by no means degenerating cells at the end of the production phase, but remain active and instrumental in the maturation of the enamel matrix.

(1) *Changes in the transitional period.*

Longitudinal sections through continuously growing teeth present the changes accompanying the close of production and the beginning of maturation as a series of regressive followed by progressive changes. The regressive changes are seen in the disappearance of the status held during production, the progressive changes in the reestablishment of a columnar epithelium.

Figs. 23 and *24* show many of the nuclei of the ameloblasts released from their attachment to the base of the cell. With this depolarization in progress, the cells become shorter and the gap toward the matrix disappears. The breaking down of the line of demarcation toward the stratum intermedium cells follows soon afterwards. The regressive changes are transitory ones and pass into a reorganization of the enamel epithelium in which it appears as a regular columnar epithelium consisting of shorter cells with the nuclei again basal.

The zone of transition with the temporary loss of cell polarity may be more or less marked. The more rapidly the epithelium loses its tall prismatic form, the more conspicuous the alteration becomes. When the epithelium is slowly reducing in height, the depolarization may escape the observer. It is, therefore, not surprising that this phase in the history of the ameloblasts has been over-

looked by all previous investigators except Carter ('18). His work on the cytomorphosis of the marsupial enamel organ gives a most elaborate description of the ameloblasts throughout tooth development. He divided the life cycle of the ameloblasts into 14 stages of which stage 6 is undoubtedly regressive in character and the 2 following stages, in which the cells return to a definite columnar form, are progressive. The temporary disorganization and the consecutive restoration of the columnar epithelium in Carter's material seem to correspond to the regressive and the progressive changes found in the teeth of the guinea pig. To compare these findings more accurately with Carter's observations is, however, not possible as the author failed to correlate his stages of the ameloblasts to the different phases of enamel development.

Since the regressive changes neutralize the structural characteristics acquired by the enamel epithelium at the beginning of amelogenesis, it is a fair conclusion that the factors which brought about these characteristics cease to act at the end of the period of production. In other words, the ameloblasts are no longer oriented toward the source of supply they began to utilize when they were isolated from the pulp by the dentin. Accordingly, the demarcation of the enamel epithelium from the stratum intermedium, the polarity of the cells and the space toward the enamel matrix are no longer maintained. In almost every case globular material accumulates in both the ameloblasts and the stratum intermedium cells (*figs. 23, 24,* and *32*). This indicates clearly that the flow of fluid into the cells from the outside has been stopped. It also shows that the end of production cannot be due to a waning activity of the cells which continue with secretion when the transfer of the secreted material is no longer possible. Soon afterward the elaboration of globular material comes to an end as no more specific substances are taken in.

It was stated above that the end of enamel production seemed to be due to a change in the environmental conditions of the ameloblasts. The question of the causes can now be formulated by inquiring into the factors which may stop the flow of fluid into the cells. It may be that the enamel matrix, after a certain period, through changes which initiate maturation, loses the hydrophilic property on which the current from the base to the secretory pole depends. This hypothesis can be tested only by a physico-chemical investigation of the maturation process, not by histological methods. Nevertheless, its presentation here seems justified as a possible clue toward the understanding of the striking regularity in the limitation of enamel production.

The reduction of the ameloblasts at the end of production cannot be due to a direct tranformation of the cell into enamel rods as has been pointed out. When the ameloblasts are reduced in form, they appear united with the stratum intermedium cells as a syncytium (*figs. 23, 24, 27, 31,* and *32*). The intercellular spaces are considerably wider than in the preceding period. The stratum intermedium cells spread over and often surround the adjacent blood capillaries. The volume of the individual ameloblasts is reduced as they lose about 50% in height and become only a trifle wider. At the same time the boundary toward the stratum intermedium disappears and the amount of intercellular fluid in-

creases (compare *figs. 26* and *27*). The nuclei of the ameloblasts are not similarly reduced, but become more plump. All these signs indicate that the reduction of the ameloblasts is due to the release of fluid into the interstitia.

Further evidence for the reversal, not only of the flow of fluid, but also of other materials at this time, is taken from the fact that the short ameloblasts and stratum intermedium cells beyond the transitional zone never contain any globular material. At the time of the reduction of the cells such material is regularly found within their cytoplasm (*figs. 23* and *24*). Since the cells cannot discharge the globules toward the secretory pole as they did during the production of the matrix, they can be eliminated only toward the periodontal tissue. Thus, the change of direction which follows the stoppage of the flow of fluid into the ameloblasts not only involves removal of water, but also emission of globular substance toward the stratum intermedium cells. As indicated by the enlarged intercellular spaces particularly between the stratum cells (*fig. 27*), the fluid is discharged into the interstices, while the globular material seems to pass across the cell bridges into the stratum intermedium cells which are often found heavily loaded with large globules. If this is true, a two-way removal from the ameloblasts would correspond to the two-way uptake during enamel production (see p. 474). The globular material must be taken soon afterwards from the stratum intermedium cells into the blood capillaries as the same cells somewhat closer to the surface are free from these substances.

The second turning point in the history of the ameloblasts, as we may call this decisive change in function, not only implies the removal of fluid and of globular material, but seems to prepare the ameloblasts for absorptive activity during the period of maturation. This is suggested by the observations on vital staining. Twenty-four to 48 hours after a single injection of trypan blue the enamel matrix is found diffusely stained when the active ameloblasts contain fine blue granules (see p. 473 and *fig. 12*). In contrast to the enamel matrix, the hard enamel remains unstained no matter how often the injections are repeated. This finding was anticipated by Ganzer (1906). After injecting sodium indigosulfate in guinea pigs, he found the "uncalcified" part of the teeth (the enamel matrix) intensely stained while the "calcified" part (the hard enamel) was unstained. Marshall (1920), who injected dianilblue RR intraperitoneally in rats stated that, "as the enamel rods grow older, and the tissue in its growth moves towards the cutting edge of the tooth, the stain fades out . . ." That is to say that during maturation a decoloration takes place. In the case of the trypan blue color loss might be due to a reduction of the dye to its leukobase. However, the same difference between enamel matrix and hard enamel is found when the vital staining has been done by alizarin red which may be changed in color but not bleached by chemical changes[5]. Thus, it is probable to conclude that the dye is removed from the enamel matrix during maturation. There is no indication of any transfer of material from the enamel matrix toward the dentin at this time, whereas, the reversal of the flow of fluid from the ameloblasts toward the stratum intermedium

[5] Oral communication from Dr. M. Massler, School of Dentistry, University of Illinois.

makes it very probable that the removal of substances from the matrix will be carried out into the same direction.

My observations concerning the reaction of the short ameloblasts and the stratum intermedium cells to vital staining are compatible with the idea of an absorptive activity of the ameloblasts during maturation. By sacrificing the animals at various intervals after a single injection of trypan blue (see p. 472), it was found that after about 4 days no dye is stored by the young ameloblasts which are entering the zone of production while the older ameloblasts have retained their stain. In other words, trypan blue is no longer in circulation, as the dye is either stored within the tissues or has been eliminated from the body. Similar results were obtained by Teploff ('25), who found that in guinea pigs incisors, 9 days after the last injection of lithium carmin, the dye disappeared from the apical end of the tooth. In contrast to the high ameloblasts, the short ameloblasts and the stratum intermedium cells contained the same amount and the same kind of trypan blue granules at all times until the end of the experiment 11 days after the injection (*fig. 14*). The granules enclosed in the cytoplasm of the short ameloblasts and stratum intermedium cells are fewer and bigger and more irregular in shape than these within the high prismatic ameloblasts. In addition, during maturation the stratum cells contain these granules regularly while in the period of production they are often completely unstained (*fig. 12*). In one of the animals, the uppermost tall ameloblasts had retained only a few blue granules on the eleventh day after the injection and yet the short ameloblasts emerging from the zone of transition, as well as the adjacent stratum intermedium cells, contained the usual amount of stained particles. It is difficult to believe that the dye found in the short ameloblasts and stratum intermedium cells is only the residue of the few granules present within the cells at the time of their transformation. It is more probable that the short ameloblasts are taking the dye from the enamel matrix when the latter loses its color. Also, the constant presence of blue particles within the stratum intermedium cells during maturation is most likely due to continuous movement of the dye from the ameloblasts toward the stratum intermedium cells which is opposite to the movement of the globular material at the beginning of this period. The opposite interpretation may appear to be true for the ameloblasts, that due to the loss of fluid the dye simply accumulates in these cells and concentrates into coarser particles. This, however, does not explain the constant presence of the granules within the stratum intermedium cells nor their size and shape, for these cells do not undergo the same change as the ameloblasts. Furthermore, the presence of the dye aggregates, during and beyond the entire period of maturation with no noticeable decrease in numbers, is another factor suggesting the continuous uptake of the dye as long as maturation lasts. This, in itself, does not eliminate the possibility that the dye in the short ameloblasts is a residue of the dye contained in the high ameloblasts at the time of their shortening, since the removal of dyes vitally precipitated in the cytoplasm will take more time than the maturation of the enamel matrix. However, this fact carries some weight if considered with all the other findings in favor of an absorptive function of the short ameloblasts. The loca-

tion of the trypan blue granules within the short ameloblasts points in the same direction. As can best be seen in a tangential section through the maturing matrix and the enamel epithelium (*fig. 13*), the granules are situated in the supranuclear zone and the pole of the cell next to the matrix contains only slightly stained and much less concentrated material. This is the distribution and concentration to be expected if the dye is absorbed from the matrix.

(2) *The function of the short ameloblasts.*

The conclusion reached regarding the function of the short ameloblasts is strongly corroborated by the finding that these cells are indispensable for the maturation of the enamel matrix. Schour, Chandler and Tweedy (1917) found that in parathyroidectomized rats abundant organic material remained within the enamel space when the enamel epithelium was damaged or missing. However, Chase was the first to state (1940), "that the presence of functional ameloblasts is essential for completion of the maturation of the enamel in rat incisors." This discovery was made in cases of vitamin B deficiency, of fluorosis and of direct traumatization of the enamel epithelium. My material of guinea pig molars in vitamin C deficiency presents quite a few analogous cases. *Figs. 28*, *29* and *30* show without further explanation the close correlation between the presence of the enamel epithelium in its reduced form and the maturation of the matrix. The alternation of hard decalcifiable enamel and enamel matrix, coinciding with the presence and absence of the enamel epithelium in *fig. 28*, is particularly convincing evidence of the function of the short ameloblasts during maturation. This function can be neither secretion of globular material nor production of ground substance as this kind of activity of the ameloblasts has ceased. Moreover, it cannot be secretion of any kind since the direction of the flow of fluid is now reversed. This point has to be stressed in order to prevent the a priori conclusion that the short ameloblasts may serve the transfer of minerals into the maturing enamel. The only conclusion we can draw from the new findings illustrated in *figs. 28*, *29* and *30* is that the ameloblasts, after the reconstruction of the columnar epithelium, take up a function different from that in the preceding period. This again supports the idea of their absorptive activity during maturation.

The new facts disprove once and for all the idea that the "reduced" ameloblasts are degenerating cells. Incidentally, these ameloblasts also react to vital staining differently from injured or dying cells, which are known to stain diffusely, instead of storing the dye in form of granules.

Finally, a more specific statement can be made concerning the extent to which the ameloblasts participate in the maturation process. In the absence of the enamel epithelium, the enamel matrix cannot complete its maturation. However, maturation does proceed to a certain stage without ameloblasts. This is proved by the considerable loss of stainability and by the appearance of structure seen in *fig. 30*, wherever the enamel epithelium is missing. What has been accomplished there without ameloblasts is a typical step toward the final change into hard enamel as shown by any section of a developing tooth at the proper stage or of

a continuously growing tooth at the corresponding levels (*fig. 31*).[6] It is, therefore, evident that the ameloblasts are responsible for but one part of the total necessary to complete maturation. The initiation of this complex process change is due to factors other than the activity of the ameloblasts.

The histological findings allow us to elaborate this conclusion. The decolorizing of the enamel matrix often begins at the dentino-enamel junction where a band of the matrix takes the character of the maturing enamel (*fig. 32*). This may occur prior to the change within the enamel epithelium so that production and maturation are overlapping. While it proves the beginning of maturation to be independent from the enamel epithelium the phenomenon indicates, on the other hand, an influence on the matrix via the dentin. In this way the histological analysis leads to a conception of the maturation of the enamel matrix as a twofold process, one part of which depends on the pulp the other on the ameloblasts. This conclusion is to be correlated to our knowledge concerning the nature of maturation.

(3) *The nature of maturation.*

Since the classical studies on amelogenesis by von Ebner, the changes observed in the maturing enamel were ascribed by the histologists to the increasing uptake of calcium on the one hand and to the loss of water and of organic material on the other. This concept has been substantiated in recent years by quantitative chemical and x-ray analyses of both hard and developing enamel (Rosebury; Le Fevre and Manly; Deakins and Volker; Glock, Mellanby, Murray and Thewlis). Of special interest is the correlation of chemical and histological investigations of developing enamel by Weinmann, Wessinger and Read (1942). According to these authors, the acid insoluble enamel matrix of permanent tooth germs of the pig contains 28 to 37% water, 22 to 38% organic material, 23.4 to 30.3% calcium and 12 to 15.3% phosphorus. The values for acid soluble enamel in different areas from the occlusal to the cervical end of the tooth were found to range between about 6.5 to 25% water, 4.0 to 9.5% organic material, 34.6 to 37.6% calcium and 18.1 to 25.5% phosphorus. The authors summarize their results thus: "calcification of enamel is characterized by a removal of moisture and organic material and an influx of mineral salts." The amount of organic material, according to this analysis, decreases from about 40% in the young enamel matrix to about 0.5 to 2% in the hard enamel. However, since the

[6] A possible objection is whether the ameloblasts might have started maturation and, afterwards, atrophied. Two counter objections are: (1) the ameloblasts are most susceptible to destruction in the period of transition from tall, prismatic to reduced (resorptive) type. In some of our cases the destruction begins at the most apical part of the short enamel epithelium, where no maturation change has taken place. Higher up the changes in stainability and in the appearance of structure indicate that maturation has proceeded as far as it goes in such cases with the ameloblasts missing from the beginning. (2) When the maturation changes are first visible they appear at the dentino-enamel junction. (Although according to careful histologic studies by Nuckolls, Saunders and Frisbie, 1942 and earlier crystallographic studies by Kitchin (J. D. Res., **17**: 274, 1938), in *normal* maturation (crystallization) the process starts at the peripheral portions of the rods. Ed.).

enamel increases in mass during the hardening process from about 1 to 3 (the specific gravity of apatite being between 3.14 and 3.22 according to Karlström ['31]) and since the volume of the enamel remains the same, the actual loss in organic material will not amount to more than 30% of the original value. Still the removal of proteins from the enamel matrix is an essential part of the complex maturation process. The question arises how the organic material may be taken from the matrix or which pathway may be used for its removal.

(4) *The pathways of substances taken in and removed from the maturing enamel matrix.*

Maturation is a dual process from the histological point of view depending on the activity of the ameloblasts and on the pulp. It is a twofold process likewise from the chemico-physical point of view as it consists of the 2 contrasting changes of uptake of minerals and of removal of water and proteins. The problem arises whether the 2 histologically distinct processes correspond to the 2 chemico-physical changes. In terms of histo-physiology one may seek the pathways of the substances taken up by and removed from the enamel matrix.

It must be remembered that the young enamel contains calcium and phosphorus from the beginning of amelogenesis (Hintzche and Baumann ['33]; Wassermann ['41]; Nuckolls ['41]). The uptake of calcium during maturation is, therefore, an additional calcification. The imbibition of the young enamel with minerals is intimately connected with its production, for the minerals appear to be stored within the stellate reticulum of the enamel organ (p. 468) and are, therefore, part of the substances transferred into the matrix by the ameloblasts. The pulp, or the odontoblasts, are not likely to contribute to the mineralization of the young enamel at a time when calcium and phosphorus are being retained by the dentin. Van Kossa sections show the dentin and the young enamel equally impregnated with silver salt which indicates about the same concentration of calcium phosphate at either side of the dentino-enamel junction. Since the uptake of calcium from the pulp on the one hand and from the stellate reticulum through the ameloblasts on the other leads to about the same concentration, no calcium will pass across the dentino-enamel junction.

After the period of production when the additional uptake of minerals into the enamel takes place the situation is totally different. First, there is no longer an afflux from the outside; secondly, the dentin production has slowed down, for most of the dentin has been produced. Thus, it is very probable, that the additional calcium will be drawn into the enamel via dentin from the pulp. In other words, the uptake of minerals into the maturing enamel is different from the uptake during the period of production, both as to the source and the pathway involved. This is no mere assumption as there is evidence that the hard enamel, as well as the maturing enamel is taking in a certain small amount of calcium throughout life. This has been shown by the aid of radioactive phosphorus (Chievitz and Hevesy; Hevesy, Holst and Krogh; Manly, Le Fevre and Bale; Hevesy and Armstrong). Our own studies by the same method on the different pathways of exchange of minerals in teeth (Wassermann, Blayney, Groetzinger and DeWitt) proved that the calcium taken into the enamel of the teeth of adult

dogs passes through the dentino-enamel junction and is drawn mainly through the pulp. When the pulp was removed and the inner surface of the dentin sealed off, only one-fifth of the normal amount of calcium entered the enamel via cementum and dentin. What is true of the additional uptake of minerals after the period of maturation will hold also during this period when the calcium requirement of the enamel is much greater and the primary source of mineral supply has already ceased to flow. In addition, circumstantial evidence can be presented. In guinea pig molars, the calcium supply in later amelogenesis cannot be the same as during the period of production. Toward the end of maturation the cells of the periodontal tissue begin to cluster and to form cartilage (*figs. 1* and *7*). The latter contains considerable quantities of calcium as can be easily demonstrated by the von Kossa method. Thus, the available calcium is kept within the periodontal tissue instead of being sent into the enamel as it was in the preceding period.

Since the additional calcification of the enamel is due to the uptake of minerals from the pulp via the dentino-enamel junction, the function of the short ameloblasts known to be indispensable for maturation must be related to the removal of fluid and of organic material from the enamel matrix. In other words, the second turning point in the history of the ameloblasts, indicated by a series of structural changes, is from the functional point of view a change in function from secretion to absorption. This has been suggested by (1) the changes within the ameloblasts and stratum intermedium cells at the beginning of maturation; (2) the decolorization of the hardening enamel after vital staining; (3) by the reaction to vital staining of both the ameloblasts and the stratum intermedium cells. It is, therefore, safe to say, that we come as close as it is possible at present to proving that the short ameloblasts are absorbing cells. In general, the ameloblasts present another example like the columnar epithelium of the intestinal wall, demonstrating the ready shifting from one direction to the other of columnar epithelial cells. The free surface of the cell acts as a membrane which serves either for the uptake or the elimination of substances, depending on changing conditions.

Finally, it may be shown from another viewpoint that the organic material cannot be removed from the enamel matrix through the dentin, but only by the action of the ameloblasts. A deficient enamel resulting from incomplete maturation, as it is shown in *fig. 30*, cannot be repaired later. The same is true of the identical "hypoplastic" enamel which occurs in mammalian and human teeth, and is characterized by a deficit in calcium and an excess in organic material (Bodecker ['41]). The calcium deficit in such parts of the enamel could be made up since calcium is being taken up by the enamel throughout life, as shown with the aid of radioactive phosphorus, but the organic material cannot be removed once the ameloblasts are gone. The changing metabolic conditions during the production and the maturation of the enamel matrix of any tooth, as they are presented in this paper, are summarized in the diagram of *fig. 6*.

(5) *Crystallization of the enamel.*

Kitchin ('33), studying the development of the enamel in ground sections by the aid of the polarizing microscope, was able to demonstrate the formation of the enamel crystals. These fundamental studies make it necessary to distin-

guish between calcification and crystallization of the enamel. We know that the young enamel in all its stages of development contains calcium. Calcification, that is incorporation of calcium or minerals in general, is not identical with the final hardening of the enamel, as the use of the term calcification seems to suggest. According to Kitchin, the definitive crystal structure is established in decalcified sections when all evidence of the enamel structure disappears, that is, at the end of the period of maturation. However, the final phase is preceded by an initial phase of crystallization. We know that not only addition of mineral,

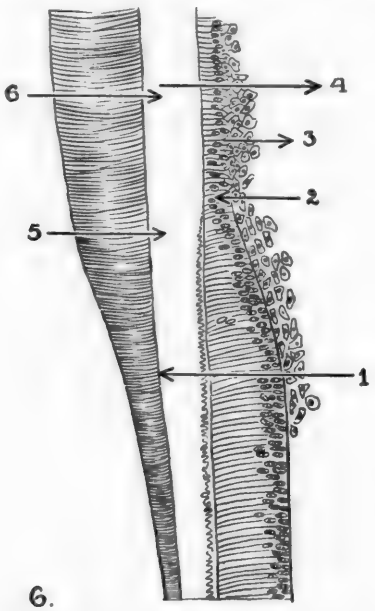

Fig. 6. Diagram to show pathways of substances taken in and absorbed from enamel matrix during period of production and maturation. Arrow 1—flow of material through the ameloblasts during production; arrow 2—stoppage of secretion; arrow 3—reversal of flow of material at the beginning of maturation; arrow 4—absorption of material from enamel matrix by the ameloblasts; arrows 5 and 6—intake of minerals by enamel matrix through dentin.

called here additional calcification, but also removal of water and organic substances is indispensable to bring about the crystallization of the enamel. How closely correlated the 2 processes are is demonstrated by the enamel dysplasia that results from incomplete maturation: insufficient removal of water and organic material renders the enamel unable to continue calcification. We do not know how the 2 changes upon which maturation depends are physico-chemically interlocked. However, we can define maturation as the changes within the enamel matrix which result from both gradual increase in mineral and gradual decrease in moisture and organic material, and culminate in the formation of enamel crystals.

SUMMARY

(1) The purpose of a histophysiologic analysis of enamel formation is to envisage the complex process as a series of changes within all the tissues concerned and to understand the causal relations of these changes with respect to initiation, maintenance and limitation of amelogenesis (p. 463).

(2) In continuously growing teeth the development of the enamel-producing tissues can be followed from the beginning to the end in a single tooth. Three stages can be distinguished: (1) the embryonic period; (2) the period of production; (3) the period of maturation. The transitional changes between the different periods are of special interest (p. 467).

(3) During the embryonic stage, which is the homologue of any mammalian tooth germ, the enamel epithelium, prior to the formation of dentin, maintains its original relationship to the pulp which is its primary source of supply. The rest of the enamel organ is supplied by the secondary or outer source of supply, viz. the periodontal tissue (p. 468).

(4) In the beginning of amelogenesis, the ameloblasts become oriented toward the stellate reticulum and the periodontal vascular mesenchyme which now become their source of supply. This is the first turning point in the history of the ameloblasts. The structural changes, accompanying the beginning of amelogenesis (*fig. 9*), are indicative of the reversal of nutrient supply of the ameloblasts.

(5) The decisive change is not due to the vascularization of the enamel organ, but to the formation of dentin which replaces the former basement membrane between the ameloblasts and the pulp, thereby separating the inner enamel epithelium from the primary source of supply (p. 470).

(6) The influx of fluid from the outside is indicated by the gap between enamel epithelium and dentin opening at the very beginning of amelogenesis. It is kept open as long as production lasts (p. 471).

(7) The abundant uptake of fluid from the outside by the ameloblasts has been demonstrated by the vital staining method (p. 473).

(8) The demarcation between enamel epithelium and stratum intermedium is another sign of absorption going on at the outer boundary of the epithelium (p. 473). When this process comes to an end, the demarcation disappears (*fig. 23*).

(9) Since the protoplasmic bridges between the ameloblasts and the stratum intermedium cells are maintained during production, the transfer of material into the ameloblasts is carried across both the cell processes and the limiting membrane (p. 474).

(10) In scorbutic animals the formation of the dentin may be entirely suppressed in certain areas. Enamel forming tissues there remain in the embryonic stage (p. 474, *fig. 15*). The causal relation of dentin and differentiation of the enamel-forming tissues are proved by this coincidence.

(11) The building up of the enamel matrix consists of (1) the secretion of globular material, (2) of the formation of a structured ground substance (p. 476).

(12) The secretion of globular material is a function of both the ameloblasts and the stratum intermedium cells.

(13) Production of globular material, without its consequent incorporation into the ground substance, leads to the accumulation of amorphous masses laid down on top of the enamel matrix (*fig. 18*). This is the result of a hypersecretion under abnormal conditions and should not be called enamel hyperplasia as has been done.

(14) The reverse disturbance, that is the formation of the ground substance without its simultaneous impregnation with globular material, reveals the structure of the ground substance (*figs. 19, 20*). The attempt was made to trace this structure back to that of the earliest ground substance in the beginning of amelogenesis (*fig. 22*). The conclusions reached do not confirm the traditional opinion that each enamel prism corresponds to one particular ameloblast (p. 478).

(15) The growth of the ground substance does not coincide with a gradual shortening of the receding ameloblasts (*fig. 5*). Thus, the theory of the direct transformation of the ameloblasts into the enamel rods does not correctly describe the formation of the ground substance, which seems to be elaborated as an exoplasmic derivative of the cells (p. 481).

(16) The cessation of production is not due to a limited life span of the ameloblasts but to environmental conditions (p. 484).

(17) Ending of production and beginning of maturation are accompanied by regressive and progressive changes in the enamel epithelium. The polarity of the ameloblasts, the space between enamel epithelium and matrix, the demarcation of the enamel epithelium toward the stratum intermedium disappear while the ameloblasts are decreasing in height (p. 484). The reestablishment of the columnar epithelium marks the new organization necessary for the maturation (*fig. 23*).

(18) Loss of water by the ameloblasts (p. 486), retention of secreted material within the cells (*fig. 23*), and soon afterward the absorption of this material, indicate that the flow of fluid is reversed in the beginning of maturation. This is the second turning point in the history of the ameloblasts.

(19) The finding that vitally stained enamel matrix decolorizes regularly during maturation suggests an absorptive activity of the ameloblasts beginning with the reversal of the flow of material.

(20) After the destruction of the short ameloblasts, the enamel matrix does not reach complete maturity which, however, proceeds to a certain stage (*fig. 30*). It follows that the ameloblasts are charged with a function which is indispensable for one phase of maturation. This function is certainly different from that in the preceding period of production (p. 488).

(21) The concept of the twofold nature of enamel maturation is confirmed by (1) the activity of the ameloblasts and (2) the results of recent chemical analyses of developing enamel. The 2 processes on which maturation depends are the uptake of minerals, in addition to those contained in the enamel matrix from the beginning, and the removal of water and organic substances.

(22) According to recent studies by the aid of radio-active phosphorus, calcium is taken in by the enamel throughout life via the dentino-enamel junction. The same pathway will be used by the maturing enamel when the flow of material from the outside ceases. The analogy is strongly supported by the observation

that the first changes typical of maturation are found at the dentino-enamel junction (*fig. 32*). The retention of calcium within the periodontal tissues in guinea pig molars at the time when maturation is not complete also points to the dentin and the pulp as the source of calcium (p. 491).

(23) Since the uptake of minerals during maturation does not depend on the ameloblasts, their indispensable function must be related to the removal of fluid and of organic substances. A further evidence for the absorptive activity of the short ameloblasts can be derived from the irreparability of the dysplasia of the enamel (p. 491).

(24) It has been found necessary to distinguish between calcification and crystallization of the enamel matrix. Crystallization, and, therefore, the final hardening of the enamel, is the result of both calcification and the removal of water, as well as of a certain amount of the organic material from the enamel. The 2 processes involved in maturation seem to be chemico-physically interlocked so that calcification cannot be completed when absorption has been stopped.

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REFERENCES TO LITERATURE

ANNEL, G., Beiträge zur Kenntnis der zahnbildenden Gewebe des Menschen und der Säugetiere, *Biolog. Unters. von Gustav Retzius*, II: 33, 1882.

BEAMS, H., AND R. KING, The Golgi apparatus in the developing tooth with special reference to polarity, *Anat. Rec.*, **57:** 29, 1938.

BLOTEVOGEL, W., Ueber den vitalen Farbstofftransport bei der Zahnentwicklung, *Anat. Anz.*, **57:** 213, 1923.

BLOTEVOGEL, W., Der vitale Farbstofftransport während der Zahnentwicklung, *Z. f. Zellen-und Gewebelehre*, **1:** 601, 1924.

BODECKER, C. F., Enamel Hypoplasia, *J. Den. Res.*, **20:** 447, 1941.

BOYLE, P. E., The effect of ascorbic acid deficiency on enamel formation in the teeth of guinea pigs, *Am. J. Path.*, **14:** 843, 1938.

CARTER, J. T., The cytomorphosis of the marsupial enamel organ and its significance in relation to the structure of the completed enamel, *Phil. Trans. Royal Soc. London* B, **208:** 271, 1918.

CHASE, S. W., Additional evidence of the keratinous nature of the enamel matrix, *J. Den. Res.*, **9:** 288, 1929.

Idem, Histogenesis of the enamel, *J. A. D. A.*, **19:** 1275, 1932.

Idem, The nature of the enamel matrix at different ages, *J. A. D. A.*, **22:** 1343, 1935.

Idem, The interrelationship of maturation and histogenesis of hypoplasia in dental enamel, *Proc. of the Dental Centennary Celebration* (Baltimore), p. 425, 1940.

CHIEVITZ, O., AND G. HEVESY, Studies on the metabolism of phosphourus in animals, *Danske Vidensk. Selsk. Biol. Medl.*, **13:** No. 9: 24, 1937.

DEAKINS, M., Changes in the ash, water and organic content of pig enamel during calcification, *J. Den. Res.*, **31:** 429, 1941.

DEAKINS, M., AND VOLKER, J. F., Amount of organic matter in enamel from several types of human teeth, *J. Den. Res.*, **20:** 117, 1941.

DIAMOND, M., AND WEINMANN, J. P., *The enamel in human teeth; an inquiry into the formation on normal and hypoplastic enamel matrix and its calcification* (Columbia University Press, New York: 1940).

v. Ebner, V., Ueber die histologischen Veränderungen des Zahnschmelzes während der Erhärtung, insbesondere beim Menschen, *Arch. f. mikr. Anat.*, **67:** 18, 1906.

Erausquin, J., Citologia del ameloblasto, *Rev. Odont. de. Buenos Aires*, **8:** 1, 1939.

Ganzer, H., Die physiologische Injektion zum Studium der Histogenese des Zahnschmelzes, *Anat. Anz.*, **28:** 436, 1906.

Glock, G. E., Mellanby, H., Murray, M. M., and Thewlis, J., A study in the development of dental enamel in dogs, *J. Den. Res.*, **21:** 183, 1942.

Gottlieb, B., und Greiner, E., Der histologische Bau der Meerschweinchenmolaren und ihres Befestigungsapparates, *Z. Stomat.*, **21:** 565, 1923.

Green, H., The development and morphology of the teeth of ornithorrynchus, *Trans. Royal Soc. London, B*, **228:** 367, 1938.

Gulat, K., Die Entwicklung der Schneidezähne der Nagetiere unter besonderer Berücksichtigung der Kapillaren, *Morph. Jahrb.*, **77:** 382, 1936.

Hampp, E. G., Mineral distribution in the developing tooth, *Anat. Rec.*, **77:** 273, 1940.

Held, H., Ueber die Bildung des Schmelzgewebes, *Z. f. mikrosk. anat. Forsch. Bd.*, **5:** 668, 1926.

Hevesy, G., Holst, J. J., and Krogh, A., Investigations on the exchange of phosphorus in teeth using radioactive phosphorus as indicator, *Danske Vidensk. Selsk. Biol. Med.*, **13:** 34, 1937.

Hevesy, G., and Armstrong, W. D., Exchange of radio phosphate by dental enamel, *J. Den. Res.*, **19:** 318, 1940.

Hinztzsche, E., und Baumann, M., Beitrag zur Histochemie der Zahnentwicklung, *Z. f. mikrosk. anat. Forsch.*, **32:** 333, 1933.

Huggins, C. B., McCarroll, H. R., and Dalberg, A. A., Transplantation of tooth germ elements and the experimental heteropic formation of dentin and enamel, *J. Exp. Med.*, **60:** 199, 1934.

Jasswoin, G., On the structure and development of the enamel in mammals, *Quart. J. of Microsc. Sc.*, **69:** 97, 1924.

Idem, De l'histologie comparative du sang et du tissu conjonctife. XII. Sur l'origine de la substance fondamentale de l'os chez les mammifères, *Arch. d'ant. microsc.*, **30:** 411, 1931.

Jump, E. B., Vascularity of the human enamel organ, *J. Den. Res.*, **17:** 505, 1938.

Idem, Colchicine method as applied to problems of growth in dental tissues, *J. Den. Res.*, **21:** 316, 1942.

Karlström, S., Physical, physiological and pathological studies of dental enamel with special reference to the question of its vitality, *Svensk Taudlaekare Tidskr.*, **24:** Suppl. No. 1, 1931.

Kitchin, P. C., Some observations on enamel development as shown in the mandibular incisors of the white rat, *J. Den. Res.*, **13:** 25, 1933.

Lehner, J., und Plenk, H., Die Zähne, in Möllendorff's *Handb. d. mikr. Anatomie*, **5:** 3, Teil (Berlin, Springer, 1936).

Manly, M. L., and Bale, W. F., The metabolism of inorganic phosphorus of rat bones and teeth as indicated by the radioactive isotope, *J. Biol. Chem.*, **129:** 125, 1939.

Marshall, J. S., An experimental study with certain "vital dyes" of the pesistent teeth of the albino rat, *J. Den. Res.*, **2:** 1, 1920.

McLean, F. C., and Bloom, W., Calcification and ossification. Calcification in normal growing bone, *Anat. Rec.*, **78:** 333, 1940.

Mummery, J. H., *The microscopic and general human and comparative anatomy of the teeth*, 2nd ed. (London, Midford: 1924).

Nuckolls, J., Lobular development and calcification in the tooth, *J. California St. D. A.*, **17:** 73 and 105, 1941.

Orban, B., H. Sicher and J. P. Weinmann, Amelogenesis, *J. A. College Dent.*, **10:** p. 13, 1943.

Orban, B., *Oral Histology and Embryology* (St. Louis, C. V. Mosby Co.: 1944).

PRENANT, A., Contribution à l'histologie de l'email dentaire, *Arch. de Morph. gén. et exp.*, **19:** 1924.

ROSEBURY, T., A biochemical study of the protein in dental enamel, *J. Den. Res.*, **10:** 187, 1930.

SANTONÉ, P., Studien über den Aufbau, die Struktur und die Histiogenese der Molaren der Säugetiere. 1. Molaren von Cavia cobaya, *Z. f. mikrosk. anat. Forsch.*, **37:** 49, 1935.

Idem, C. Ricerche sulla structura dele organo dello smalto, *La stomatologia*, **33:** 1, 1935.

SAUNDERS, J. B. DE C. M., NUCKOLLS, J., AND FRISBIE, H. E., Amelogenesis a histologic study of the development, formation and calcification of the enamel in the molar teeth of the rat. *J. Am. Coll. Dent.*, **9:** 1, 1942.

SCHOUR, I., CHANDLER, S. B., AND TWEEDY, W. R., Changes in the teeth following parathyroidectomy., *Amer. J. Path.*, **13:** 945, 970, 1937.

SICHER, H., Tooth eruption. The axial movement of continuously growing teeth, *J. Den. Res.*, **21:** 201, 1942.

VON SPEE, Ueber die ersten Vorgänge der Ablagerung des Zahnschmelzes, *Anat. Anz.*, **2:** 89, 1887.

STEIN, T. B., The "physiological time" of the ameloblast and its relationship to the formation of the enamel, *D. Cosmos*, **76:** 445, 1934.

STUDNIČKA, F. K., Ueber die Histognenese der Schmelzschicht der Säugetierzähne, *Anat. Anz.*, **50:** 225, 1917.

Idem, Die Organisation der lebendigen Masse in *Möllendorff's Handb. d. microsk. Anatomie*, **2:** 1 Teil: 421, 1929.

TEPLOFF, I., Ueber die Rückbildung der intravitalen Carminablagerung bei Kaninchen, *Z. f. d. ges. exp. Med.*, **52:** 653, 1926.

WASSERMANN, F., Wachstum und Vermehrung der lebendigen Masse, in *Möllendorff's Handb. d. mikr. Anat.*, **1:** 2. Teil, 1929.

Idem, Enamel formation under normal and experimental conditions, *J. Den. Res.*, **20:** 254, 1941.

WASSERMANN, F., BLAYNEY, J. R., GRÖTZINGER, G., AND DEWITT, I. G., Studies on the different pathways of exchange of minerals in teeth with the aid of radioactive phosphorus, *J. Den. Res.*, **20:** 389, 1941.

WEIDENREICH, F., Ueber den Schmelz der Wirbeltiere und seine Beziehungen zum Zahnbein, *Z. f. Anat. und Entw. Gesch.*, **79:** 292, 1926.

WEINMANN, J. P., WESSINGER, G. D., AND REED, G., Correlation of chemical and histological investigations on the developing enamel, *J. Den. Res.*, **21:** 171, 1942.

WELIKANOWA, M., Zur Frage der Histognese der Nasmyth'schen Membran., *Dtsch. Mtschr. f. Zahnheilk.*, **46:** 1073, 1928.

Abbreviations for figs. 7 to 32

a, ameloblasts; a b, alveolar bone; a emb, ameloblasts embryonic; a act, ameloblasts activated; act, level of activation; a r, reduced ameloblasts; b m, basement membrane; c, or cart, cartilage; cap, capillary; c b, cytoplasm bridges between ameloblasts and stratum intermedium cells, d, dentin, dem, line of demarcation between enamel epithelium and stratum intermedium; dej, dentino-enamel junction; de, decalcified enamel; e, enamel; e h, hard enamel; e gr, enamel ground substance; en ep, enamel epithelium; en m, or en mx, enamel matrix; en org, enamel organ; en sp, enamel space; gl, globular material; gr, ground substance; h, honeycomb structure of the enamel matrix; o, or od, odontoblasts; ost, osteoblastlike cells; per t, periodontal tissue; sp, space between enamel epithelium and dentin; st int, stratum intermedium; st r, stellate reticulum; t, Tomes' processes; t b, terminal bars; vac, vacuoles; v, level of vascularization.

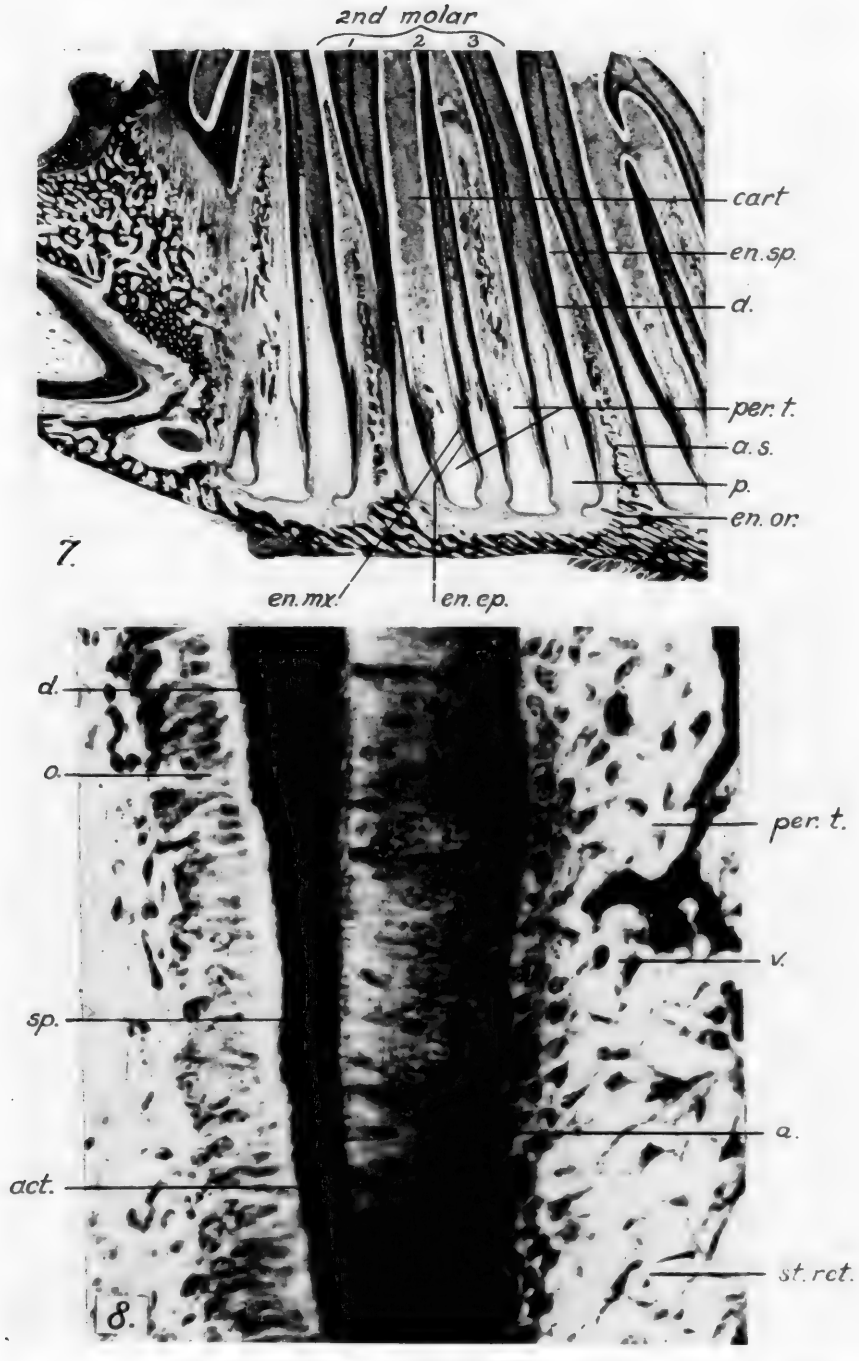


Fig. 7. Longitudinal section through lower jaw of normal guinea pig. Shows 3 lamellae of 2nd molar separated by periodontal tissue. 10×.

Fig. 8. From Azan stained, longitudinal section at level of beginning activity of ameloblasts. Receding nuclei, opening of gap between enamel epithelium and dentin Level of vascularization farther toward occlusal end than level of beginning activity. Phot. with Zeiss Oil Imm. 90×. Leitz Periplan. Oc. 12×, with Leica camera.

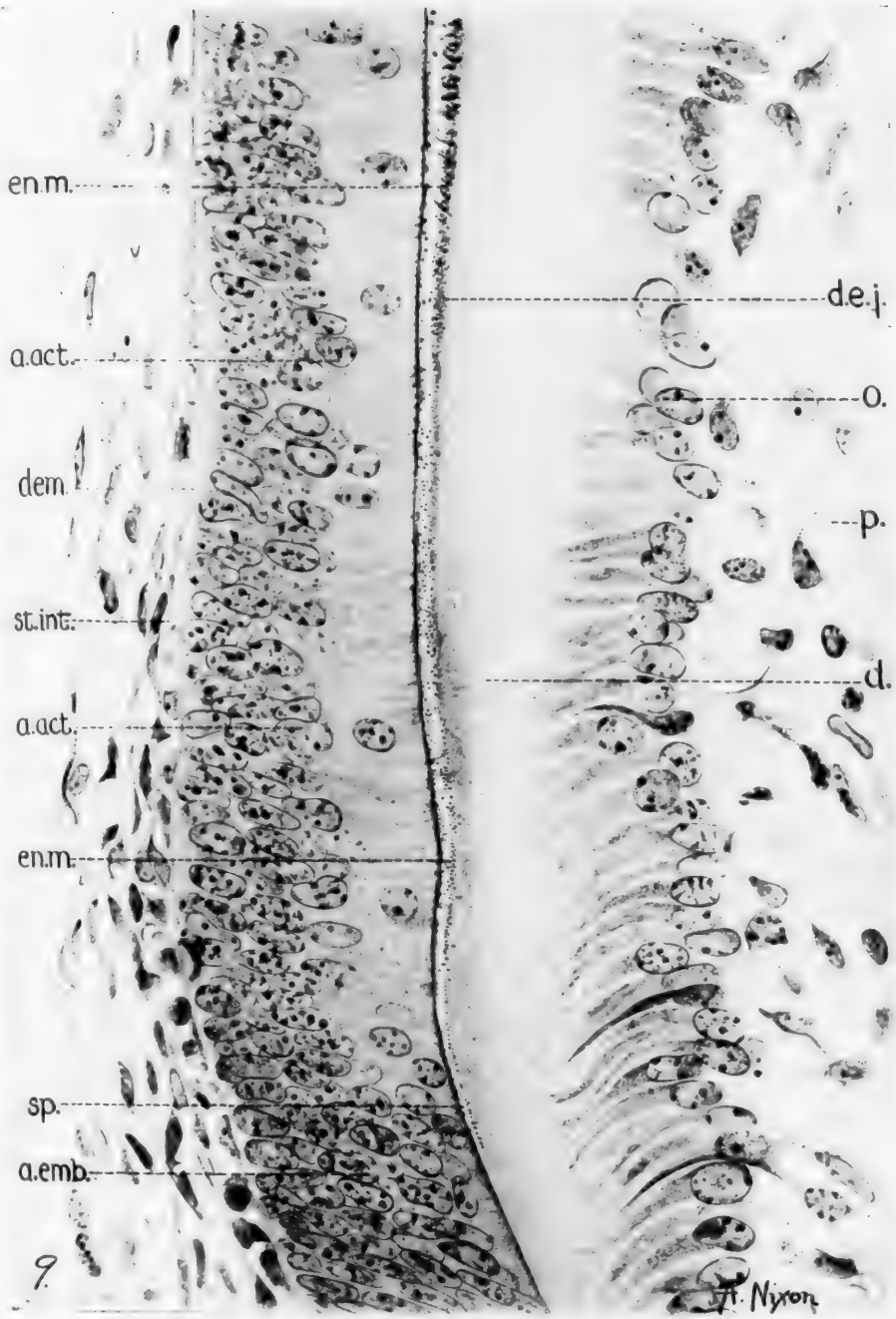


Fig. 9. From longitudinal section of tooth of normal guinea pig. Hematox. Eosin stain. Drawn with 2mm Spencer lens Oil Imm., Oc. 10×, table height. Transitional zone between embryonic and productive stage of ameloblasts. Opening of space between enamel epithelium end dentin. Receding of nuclei of ameloblasts. Demarcation of enamel epithelium toward stratum intermedium. The first enamel material laid down at prospective dentino-enamel junction in form of fine granules.

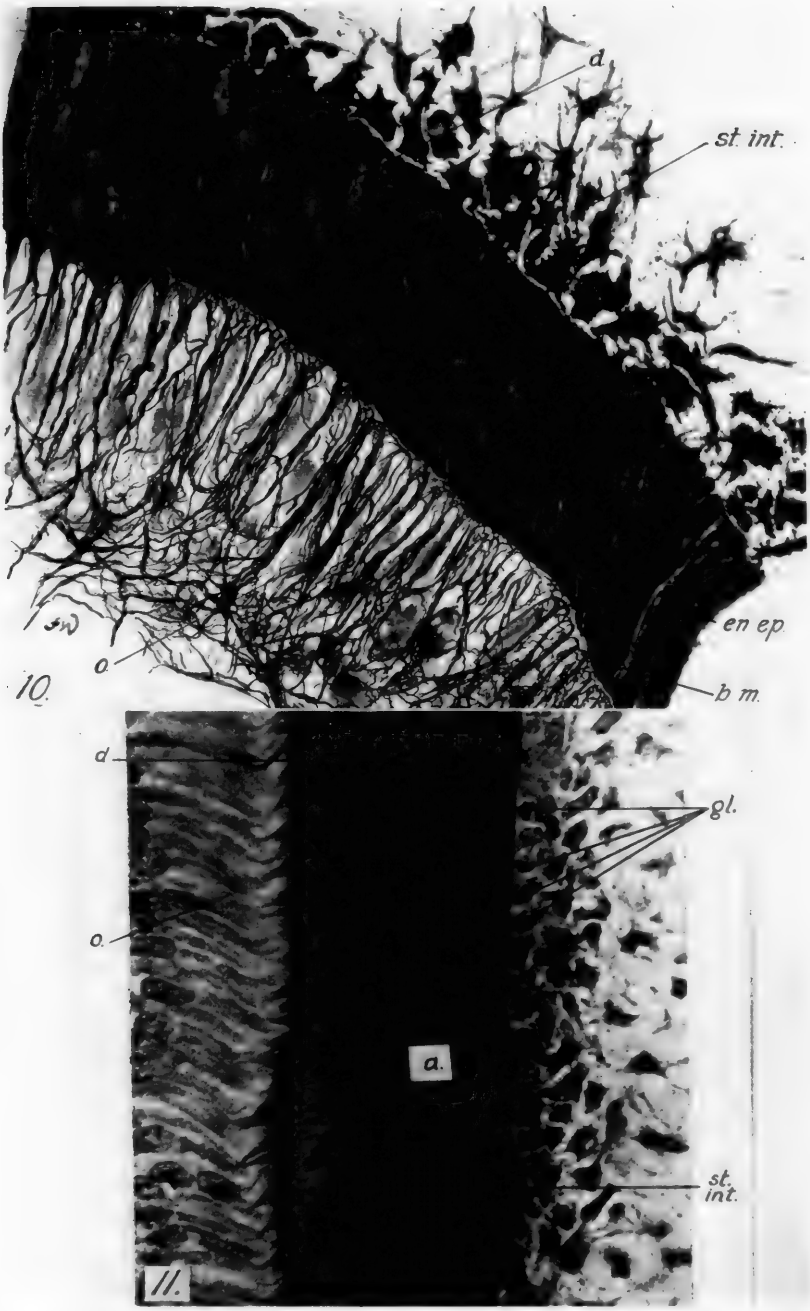
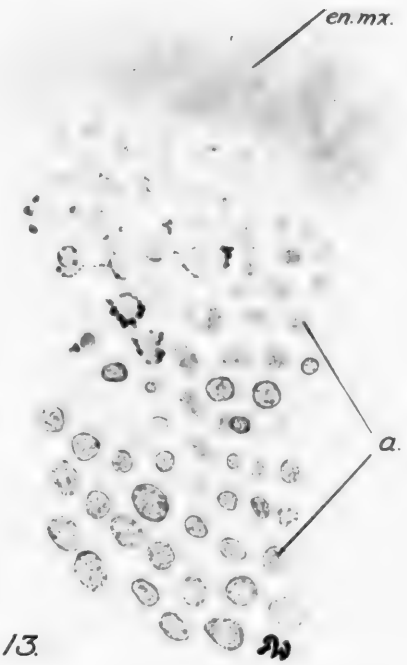


Fig. 10. Embryonic enamel epithelium shortly before activation. Basement membrane develops gradually into dentin. Bielschowsky silver stain. Drawn with Bausch and Lomb Apochr. 47. 5×, Oc.12×, table height.

Fig. 11. Level of activation of ameloblasts. Stratum intermedium cells contain globular material. Phot. with Zeiss. Oil Imm. 90×, Leitz Periplan. oc. 12×, with Leica camera, about 1000×.



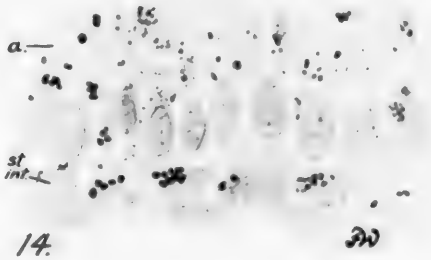


Fig. 12. Semi-diagrammatic drawing of region shown in *fig. 9* as it appears 24 hours after trypan blue injection in normal guinea pig. Trypan blue granules begin to be present in ameloblasts at time of their activation. Embryonic ameloblasts are unstained.

Fig. 13. From oblique section through inner enamel epithelium in period of maturation after single trypan blue injection. Within space between enamel matrix and nuclei of ameloblasts supranuclear parts of ameloblasts are seen in cross-sections. The trypan blue is located in this part of cell. Drawn with Bausch and Lomb Oil Imm. 47.5×, oc. 10×, table height.

Fig. 14. Short ameloblasts and stratum intermedium cells 11 days after a single injection of trypan blue. Drawn as *fig. 13*.

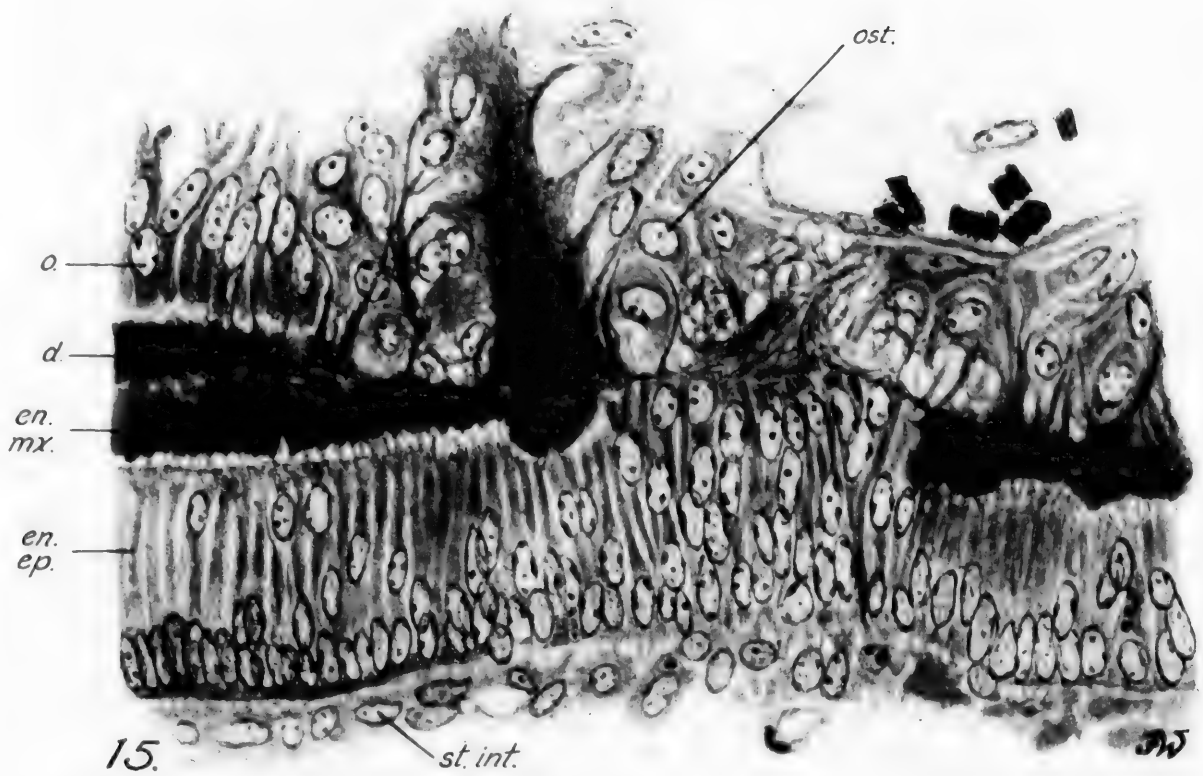


Fig. 15. From Azan stained longitudinal section of tooth of scorbutic guinea pig. Notice coincidence of interruption of dentin formation and of total interruption of enamel production. Inner enamel epithelium remained undifferentiated; no demarcation between epithelium and stratum intermedium. Drawn with Bausch and Lomb Apochr. 47.5, oc. 10×, table height.

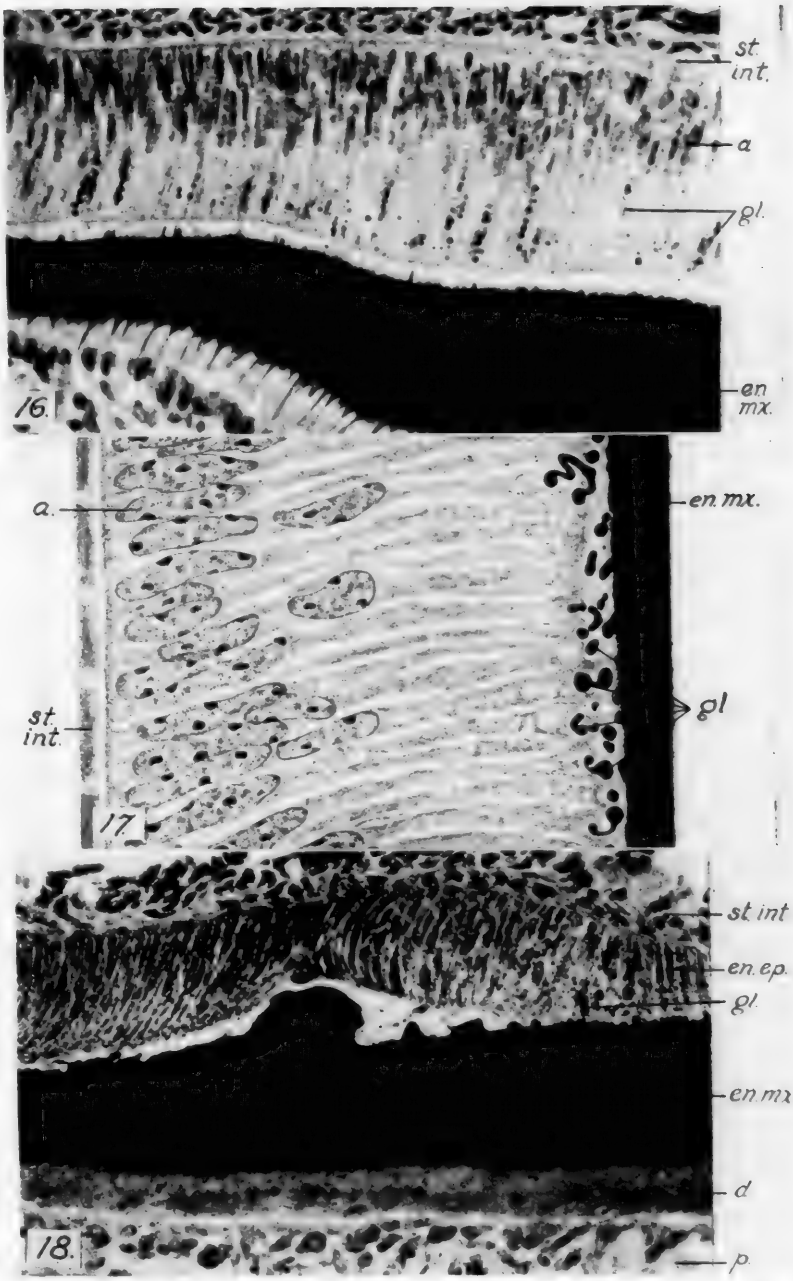


Fig. 16. From Azan stained section. Retention of globular material within ameloblasts. 530×.

Fig. 17. From Azan stained section of tooth of scorbutic animal. Globular material during emission. Drawn with Bausch and Lomb Oil Imm. 90×, oc. 10×, table height.

Fig. 18. From scorbutic animal. Enamel epithelium separated from enamel matrix. Additional globular material emitted into space of epithelial fold. Inclusion of globular material within dentin. Globules within ameloblasts. 530×.

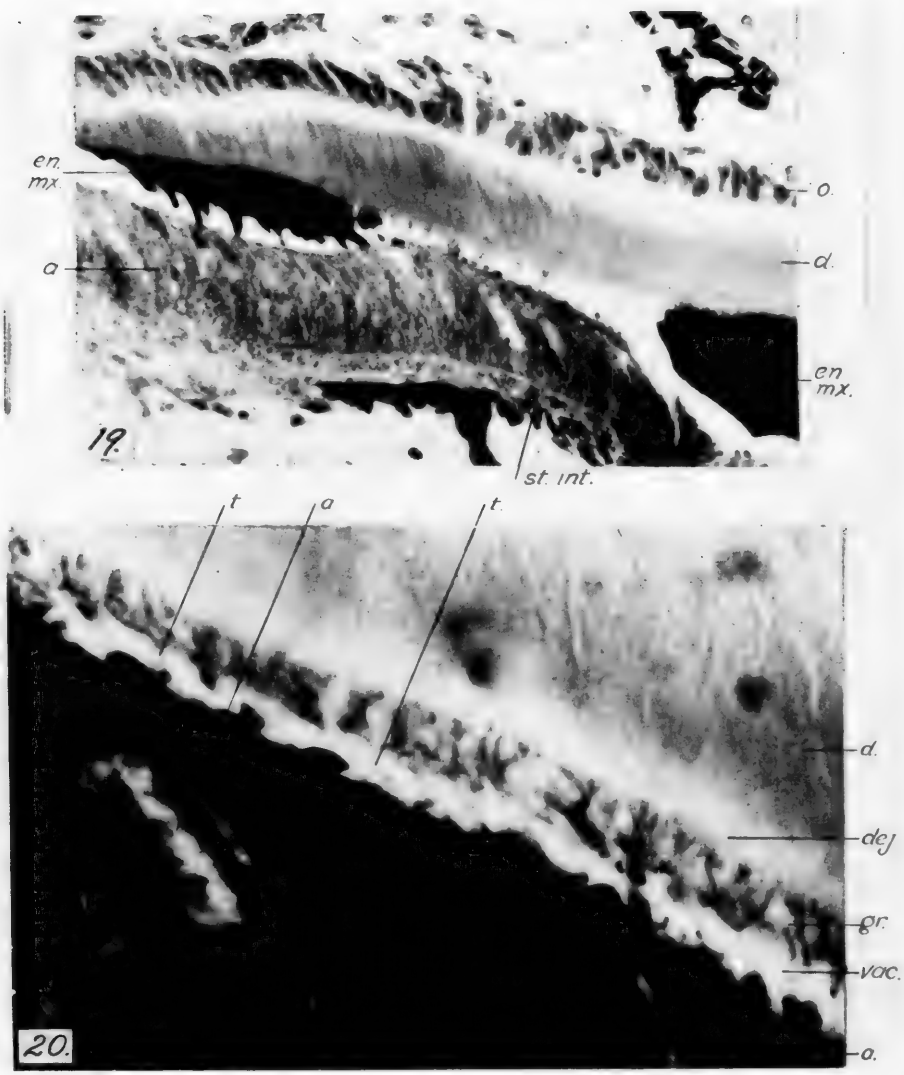


Fig. 19. From scorbutic animal. Enamel production appears to be interrupted in an area where dentin is well developed. 225×.

Fig. 20. Detail of *fig. 19* in high magnification. Where enamel production appears to be interrupted in *fig. 19* fibrillous material is seen between surface of enamel epithelium and dentin. This material is continuous with Tomes' processes and seems to consist of fan shaped parts into which the Tomes' processes spread. Globular material is withheld from this material which is groundsubstance of animal matrix. 2250×.

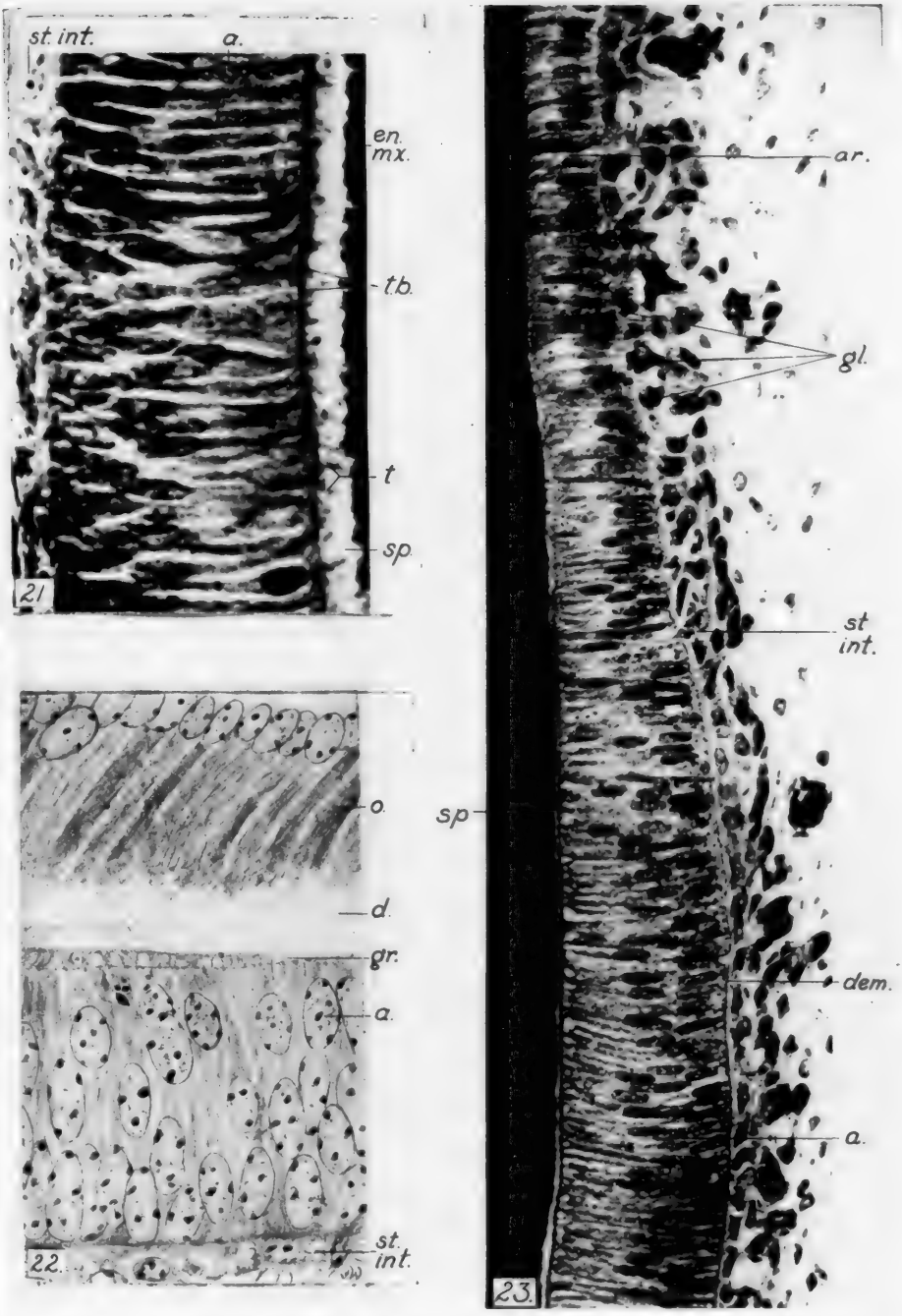


Fig. 21. Ameloblasts during period of production. Notice Tomes' processes and vacuoles within space between surface of enamel epithelium (terminal bars) and enamel matrix. 1650×.

Fig. 22. Ameloblasts in beginning of activity. Nuclei are just receding. Earliest ground substance is being produced. Notice increasing width of space between surface of epithelium (terminal bars) and dentin. Stainable material is not yet present but for few granules. Drawn with Bausch and Lomb Oil Imm. 90×. oc.12.5, table height.

Fig. 23. Zone of transition between period of production and of maturation of enamel matrix. Ameloblasts become reduced in height. Notice temporary depolarization of ameloblasts. Space between enamel epithelium and enamel matrix and demarcation of enamel epithelium from stratum intermedium disappear. Phot. with Zeiss Oil Imm. 90×, Leitz periplan. oc. 12× with Leica camera in 4 serial pictures which were put together to present survey of this region at magnification of about 1000×.

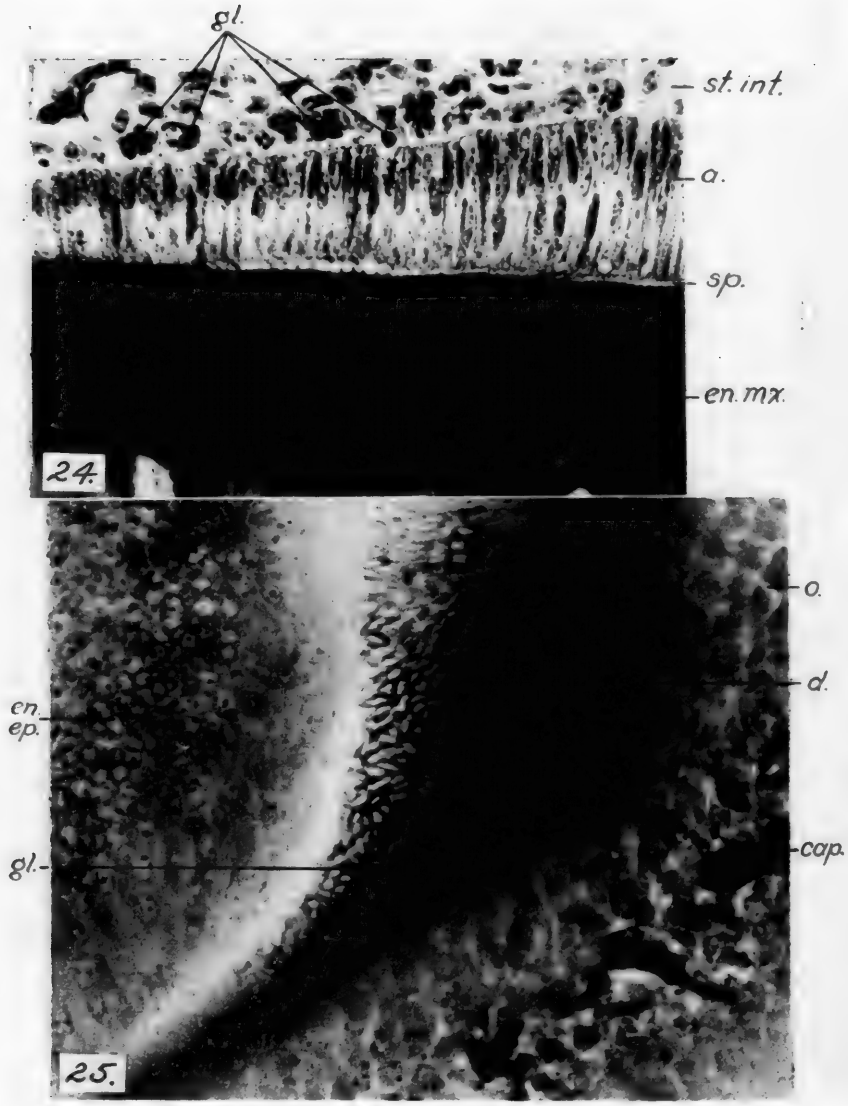
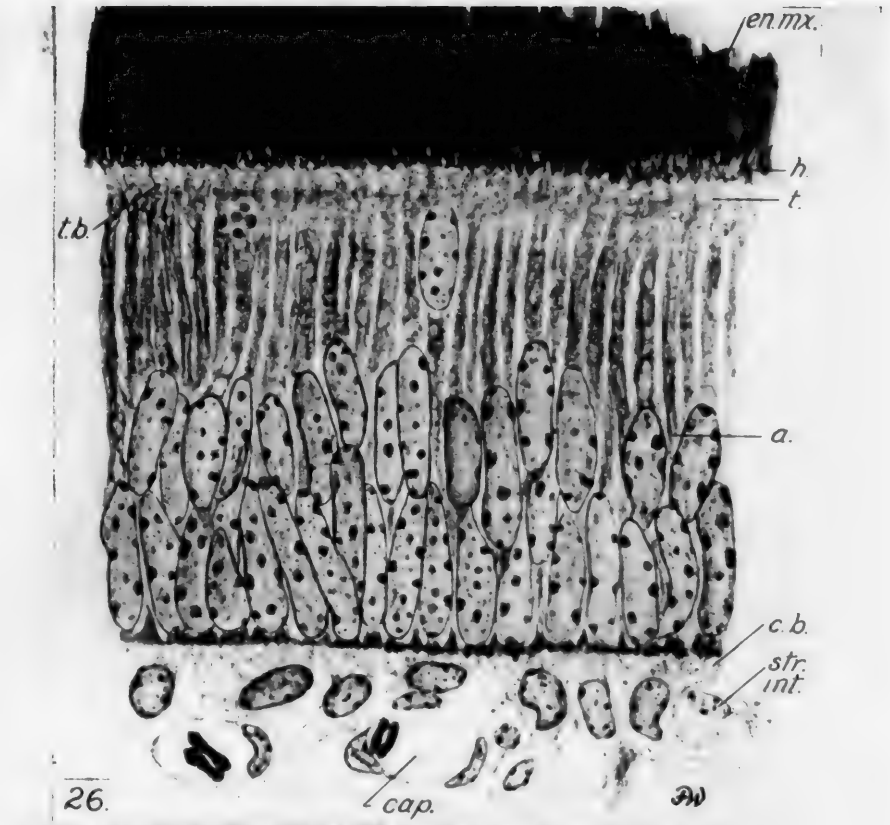


Fig. 24. Zone of transition between periods of production and of maturation. Globular material appears at end of enamel production within both ameloblasts and stratum cells. 225×.

Fig. 25. Oblique section through enamel epithelium, early enamel matrix and dentin. Stainable material of enamel matrix is seen from above. It is laid down upon dentin not in continuous layer but in pattern of network. Azan stain. By using blue filter red stained enamel material was made to appear dark on grey background of blue stained dentin. Phot. with Zeiss Oil Imm. 90×, Leitz Peroplan. oc. 8× with Leica camera.



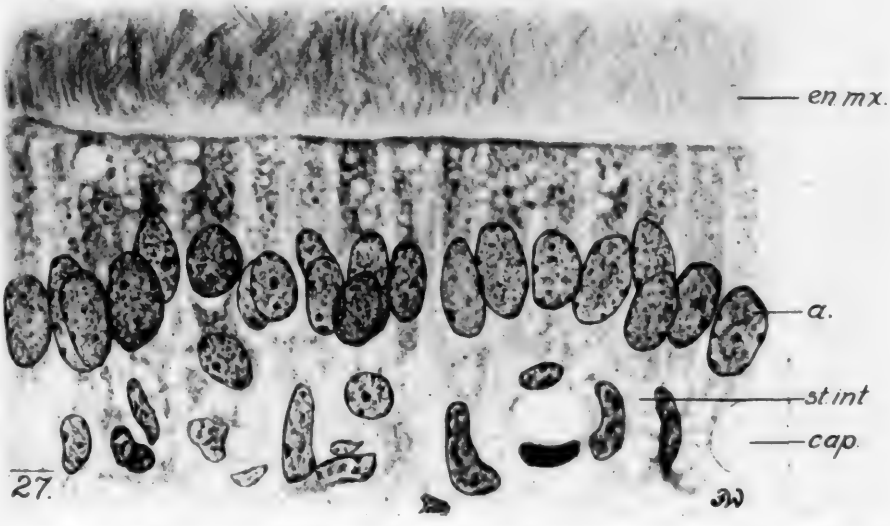


Fig. 26. Tall ameloblasts of normal guinea pig from Azan stained section. Drawn with Bausch and Lomb Oil Imm. 90×, oc. 12.5×, table height.

Fig. 27. Short ameloblasts of same animal in same magnification as ameloblasts in *fig. 26*. Notice loss of height of cells without change in width and in size of nuclei. No demarcation between ameloblasts and stratum intermedium cells. Each kind of cell forms syncytium. Intercellular spaces between stratum cells are wider than in preceding period in *fig. 26*. Cells surround capillaries. No space between enamel epithelium and enamel matrix. Latter is losing stainability and shows fibrillous structure.

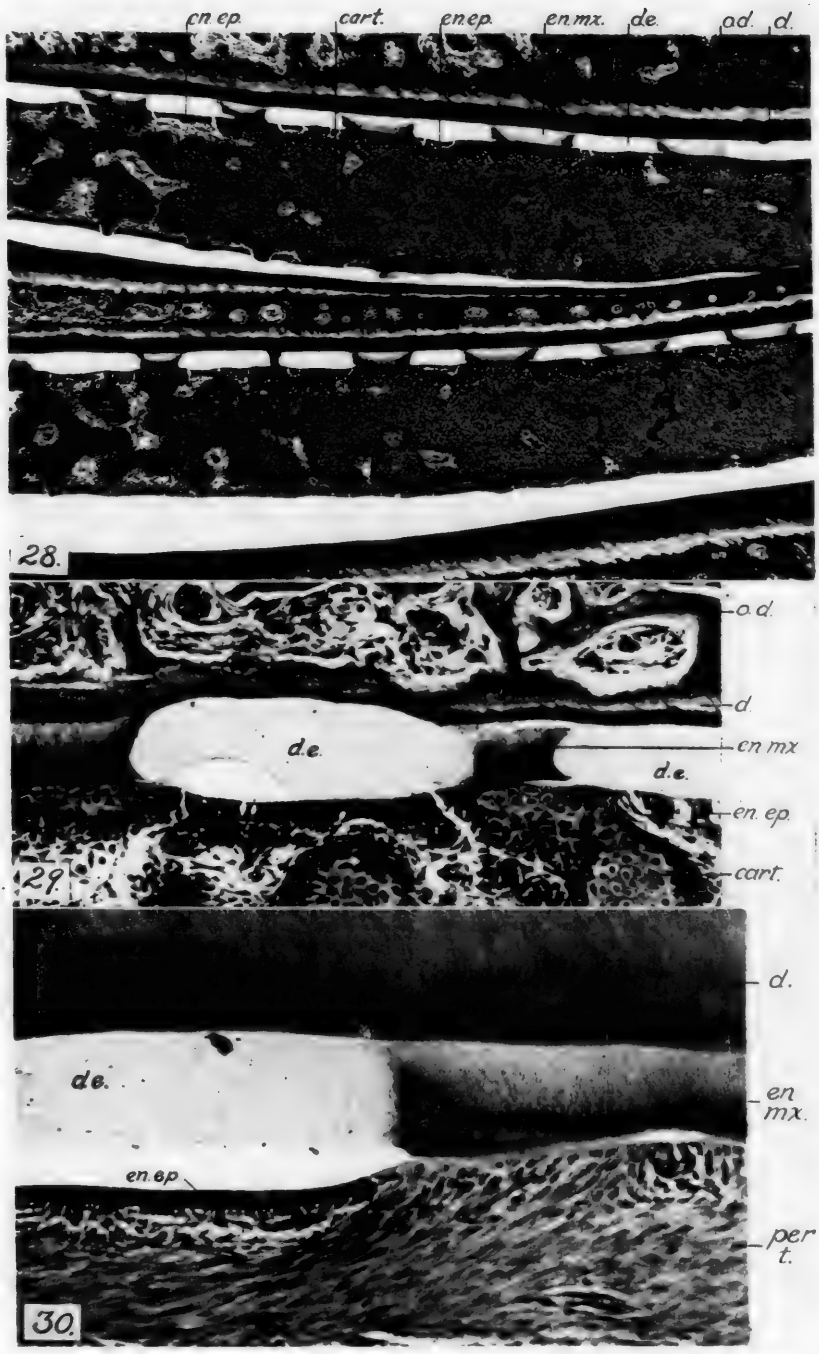
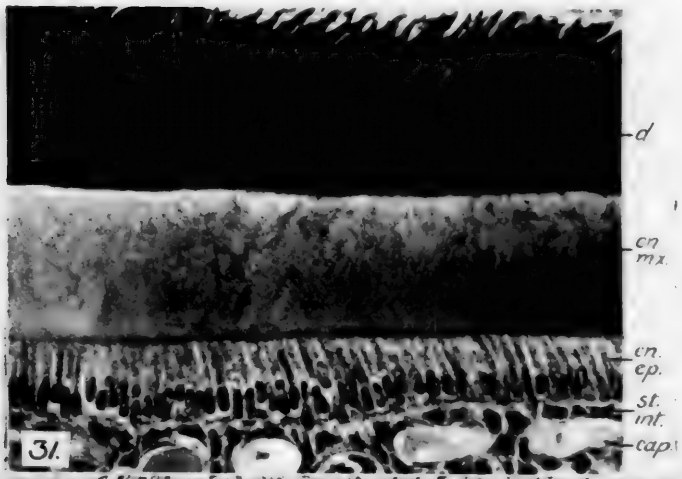


Fig. 28. Longitudinal section of tooth of scorbutic animal showing 1 of lamellae with pulp, dentin and enamel space. Periodontal cartilage fills folds between lamellae of tooth. Enamel matrix alternates with decalcifiable enamel depending on absence or presence of enamel epithelium. 58×.

Fig. 29. Enamel matrix is prevented from completing maturation where ameloblasts are absent. 225×.

Fig. 30. Demarcation of decalcifiable enamel toward enamel matrix corresponds with end of enamel epithelium. 225×.



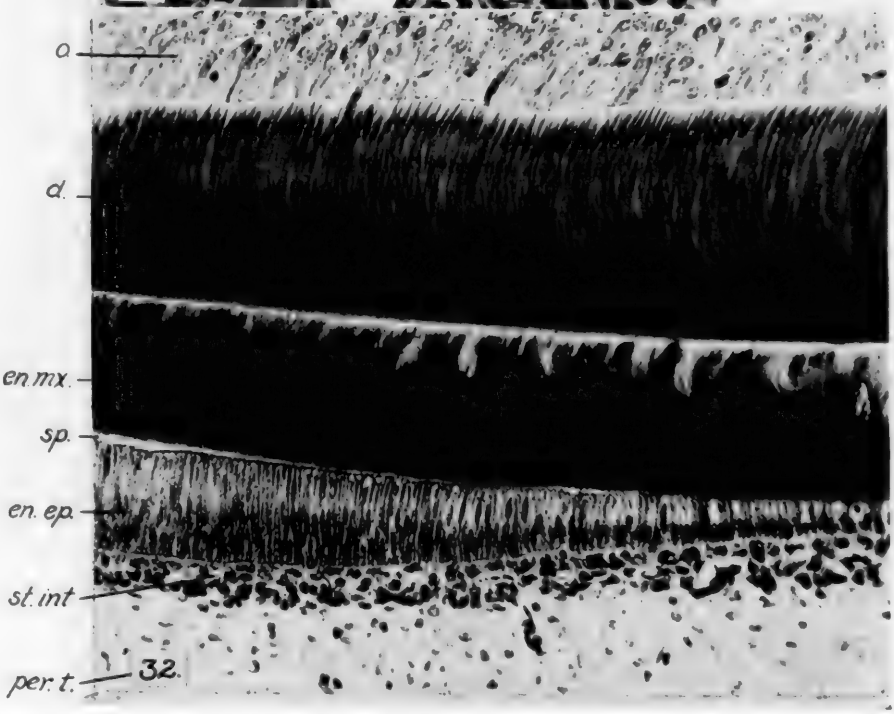


Fig. 31. Enamel matrix in maturation proceding from right to left side. Loss of stainability and appearance of structure. 530×.

Fig. 32. Zone of transition between production and maturation of enamel matrix. Typical change of enamel matrix begins at dentino-enamel junction. Phot. with Bausch and Lomb Fluoride Oil Imm. 40×, Leitz Periplan. Oc. 6×, with Bantam Camera.

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Total membership as of Dec. 1, 1944—628.

III. BOOKS RECEIVED, 1944

American Dental Association, *Accepted Dental Remedies*, 10th Ed. (Chicago, Am. D' Assoc.: 1944), pp. 306.

Bodecker, C. F., *Fundamentals of Dental Histology and Embryology* (New York, Columbia University Press: 1944).

Darlington, C. G., Wilson, G. W., Waldron, C. W., Tylman, S. D., Moore, G. R., and Miller, H. C., *The 1943 Year Book of Dentistry* (Chicago, Year Book Publishers: 1943) pp. 708.

Fry, K. W., Shepherd, P. R., McLeod, A. C. and Parfitt, G. J., *The Dental Treatment of Maxillo-Facial Injuries* (Philadelphia, Lippincott Co.: 1944).

Massler, M., and Schour, I., *Atlas of the Mouth* (Chicago, American Dental Association 1944) 49 Plates.

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[1]Abbreviations used: I. A. D. R.—International Association for Dental Research; J. D. R.—Journal of Dental Research.
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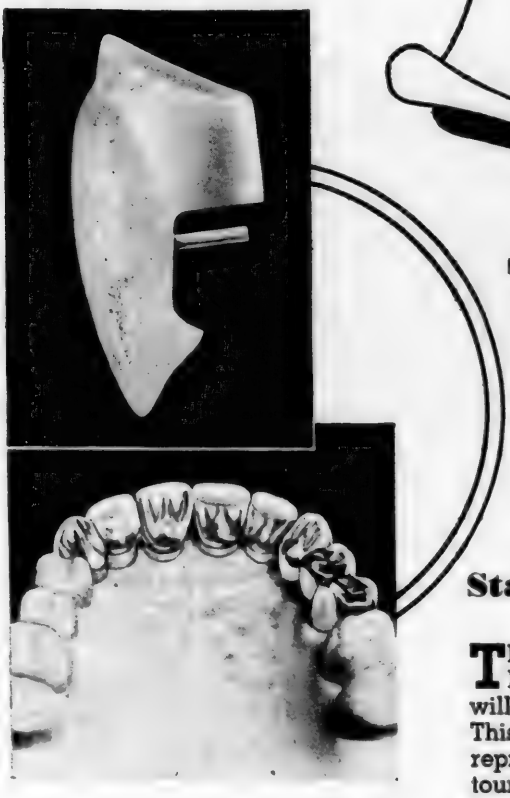

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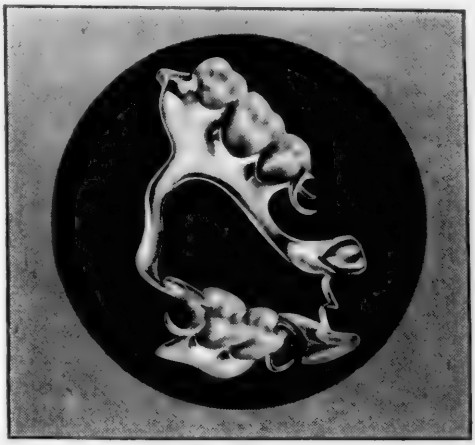

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